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THE LANGE TEST*

I. THE INFLUENCE OF PARTICLE SIZE AND HYDROGEN-ION CONCENTRATION OF GOLD SOLS UPON LANGE TEST READINGS ON PARETIC SPINAL FLUIDS

P. K. GLASOE AND C. H. SORUM
MADISON, WIS.

INTRODUCTION

THE Lange test[1] for the clinical diagnosis of certain diseases of the central nervous system, such as paresis, tabes, syphilis, encephalitis, poliomyelitis and meningitis, is not as widely used as its potential possibilities would seem to warrant. This limited use is doubtless due, in part, to the fact that gold sols of the exact quality demanded by the test are extremely difficult to prepare. Furthermore, so little is known definitely about the mechanism of the test and the specific role played by each of the reactants that irregularities that appear when the test is used are not readily interpreted.

The Lange test is used regularly in the Clinical Laboratory at the State of Wisconsin General Hospital with gratifying success. It is regarded as a definite aid in diagnosis and as a valuable means of following the progress of several of the above-mentioned diseases. Its success has been such as to warrant the inception of a program of research, having for its objective a detailed critical study of the test.

The present paper summarizes the results of a series of studies on the influence of particle size and hydrogen-ion concentration of the gold sol on test readings on paretic fluids.

*From the Department of Chemistry, University of Wisconsin, in collaboration with the Clinical Laboratory of the State of Wisconsin General Hospital, Madison.
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PREPARATION OF GOLD SOLS

The water used in the preparation of gold sols was purified by distilling it twice in a Pyrex still from a dilute solution of potassium permanganate. Using this water, the following solutions were made up:

(1) 1 per cent chloraurie acid, by dissolving one 15 grain ampoule of Mallinckrodt's $HAuCl_4 \cdot 3H_2O$ in 100 c.c. of water.

(2) 2 per cent potassium carbonate, by dissolving 10 gm. of Mallinckrodt's anhydrous potassium carbonate in water to make 500 c.c. of solution.

(3) 1 per cent formaldehyde, by dissolving 13.5 c.c. of Mallinckrodt's 37 per cent formalin in water to make 500 c.c. of solution.

(4) 0.01 M resorcinol, by dissolving 1.1 gm. of resorcinol in 1000 c.c. of water.

(5) 0.2 per cent chloraurie acid, by diluting 20 c.c. of 1 per cent chloraurie acid with 80 c.c. of water.

(6) 0.4 per cent potassium carbonate, by diluting 20 c.c. of 2 per cent potassium carbonate with 80 c.c. of water.

(7) 0.2 per cent formaldehyde, by diluting 20 c.c. of 1 per cent formaldehyde with 80 c.c. of water.

A "primary" nuclear sol is prepared as follows:

One hundred cubic centimeters of the cold distilled water are mixed with 5.0 c.c. of 0.4 per cent potassium carbonate and 5.0 c.c. of 0.01 M resorcinol. To this mixture are added 5.0 c.c. of 0.2 per cent chloraurie acid, slowly and with constant stirring. The reduction takes place immediately, an amber-colored sol being formed. When preserved in well-stoppered Pyrex containers, this primary nuclear sol is unchanged over a period of six months.

A "secondary" nuclear sol is prepared as follows:

A mixture of 5 c.c. of 0.4 per cent potassium carbonate and 5 c.c. of 0.2 per cent chloraurie acid is heated just to boiling. One hundred cubic centimeters of cold distilled water are then added. Ten cubic centimeters of the primary nuclear sol are added to this solution and the mixture stirred. Two cubic centimeters of 0.2 per cent formaldehyde are stirred into this mixture and the solution allowed to stand at room temperature until the sol is formed. When preserved in well-stoppered Pyrex containers, this sol is unchanged over a period of six months.

The final sol for use in the test is prepared as follows:

A mixture of 3.5 c.c. of 2 per cent potassium carbonate and 5 c.c. of 1 per cent chloraurie acid is heated just to boiling. Four hundred and eighty cubic centimeters of cold water are added. Into this mixture are stirred 5 c.c.* of the secondary nuclear sol, followed by 2.5 c.c. of 1 per cent formaldehyde. On standing at room temperature an orange-red sol forms slowly.

*The volume of "secondary" nuclear sol may be varied, as noted later in this paper.

INFLUENCE OF THE AVERAGE PARTICLE SIZE OF GOLD SOL ON TEST READINGS

By varying the amount of "secondary" nuclear sol used in the preparation of the final sol, the average particle size can be varied in the manner represented in Table I. Particle size was determined by direct count with the ultramicroscope.

TABLE I

VARIATION OF PARTICLE SIZE WITH VOLUME OF "SECONDARY" NUCLEAR SOLUTION

C.C. OF "SECONDARY" NUCLEAR SOLUTION	AVERAGE PARTICLE SIZE IN MILLIMICRONS
1.25	43
2.5	41
5.0	36
10.0	30
15.0	23.5

It is to be noted that, as the volume of nuclear solution increases, the average particle size decreases. Sols of large particle size were a murky amber yellow, sols with intermediate particles were a clear orange yellow, and sols with very small particles were a brilliant wine red.

Sols of various average particle sizes and constant pH hydrogen-ion concentration were used in Lange tests with samples of spinal fluid from paretic patients. Four sets of test readings, selected at random from a large number taken, are summarized in Tables II to V. All readings in a given table were obtained with the same sample of spinal fluid.

These results all indicate that, for a given pH, as the average particle size of the gold sol increases, the Lange test shows a greater degree of

TABLE II

PARETIC FLUID "A"

pH of Sols = 8.0

AVERAGE PARTICLE SIZE IN MILLIMICRONS	TEST READING
40	5555544321
38	5555543200
35	5555443100
33	3334442100
30	2223421000
23.5	1122100000
17	1122210000
10	0012310000

TABLE III

PARETIC FLUID "B"

pH of Sols = 7.1

AVERAGE PARTICLE SIZE IN MILLIMICRONS	TEST READING
42	5555555321
41	5555554321
36	5555554210
30	5555543200
23.5	5555532100
17	3344442100
10	1123110000

TABLE IV

PARETIC FLUID "L"

pH of Sols = 7.8

AVERAGE PARTICLE SIZE IN MILLIMICRONS	TEST READING
44	5555555321
39.5	5555554321
36	5555544211
30	5555543100
23.5	5555321000
17	2334442100
10	1221110000

TABLE V

PARETIC FLUID "AK"

pH of Sols = 6.3

AVERAGE PARTICLE SIZE IN MILLIMICRONS	TEST READING
40	5555555532
38	5555555422
37	5555555311
30	5555554220
23.5	5555543200
17	5555322100
10	4554221000

precipitation; as the average particle size decreases, the degree of precipitation decreases. In other words, as the average particle size increases, the sensitivity of the sol in the Lange test increases; as the average particle size decreases, the sensitivity decreases. It is thus evident that the gold sol must have the correct average particle size to be satisfactory for use in the Lange test.

THE INFLUENCE OF THE HYDROGEN-ION CONCENTRATION OF GOLD SOL ON TEST READINGS

The pH of the gold sol used in this study can be varied: (1) by varying the amount of potassium carbonate used in its preparation, (2) by varying the amount of formaldehyde used in its preparation, (3) by allowing the sol to stand exposed to the air, (4) by direct addition of dilute hydrochloric acid to the sol, (5) by direct addition of potassium carbonate solution to the sol. Using these five methods, sols of constant particle size and varying pH were made up. These sols were then used in Lange tests with spinal fluid from paretic patients. The results are given in Tables VI to XII.

TABLE VI

PARETIC FLUID "C"

Particle Size of Sols = 45 Millimicrons

pH OF SOL	TEST READING
7.57	3555432100
7.27	5555432000
7.01	5555531000
6.83	5555543100
6.63	5555553100
6.49	5555554200

TABLE VII

PARETIC FLUID "D"

Particle Size of Sols = 42 Millimicrons

pH OF SOL	TEST READING
7.67	3444310000
7.32	5554321000
6.92	5555432100
6.50	5555543210
6.34	5555554300
6.10	5555554310

TABLE VIII

PARETIC FLUID "E"

Particle Size of Sols = 37.5 Millimicrons

pH OF SOL	TEST READING
7.56	2233321000
7.21	3334321000
6.81	5554442100
6.40	5555432100
6.01	5555543210

TABLE IX

PARETIC FLUID "F"

Particle Size of Sols = 38.5 Millimicrons

pH OF SOL	TEST READING
7.37	1233100000
6.88	3333100000
6.53	5554210000
6.05	5554321000
5.72	5555443210
5.54	5554320000
5.10	5555421000
4.96	5555543210
4.38	5555554333

TABLE X

PARETIC FLUID "G"

Particle Size of Sols = 27 Millimicrons

pH OF SOL	TEST READING
8.21	2223210000
7.15	5333210000
6.57	5554210000
6.12	5554310000
5.71	5554321000
5.64	5555542100
5.36	5555421000
5.10	5554210000
5.06	5554431000
4.56	5555543100

The results show very definitely that, as the pH of the sol decreases, its sensitivity in the Lange test increases. It is thus obvious that the pH of a gold sol must be carefully controlled if it is to be satisfactory for use in the Lange test.

DISCUSSION

It is to be noted that pH and particle size may exert a compensating effect in producing a gold sol suitable for use in the Lange test. Thus, whereas increase in particle size gives a more sensitive sol, increase in pH gives a more stable sol. There are, theoretically, an unlimited number of pH-particle size combinations, all of which give a sol of the same sensitivity. Practically, however, there is a limit to these combinations because if the particle size goes above 40 millimicrons, the sols are too murky to be suitable; if the pH goes below 5, the readings tend to become erratic (see Tables XI and XII).

TABLE XI

PARETIC FLUID "H"

Particle Size of Sols = 24 Millimicrons

pH OF SOL	TEST READING
7.40	3344321000
6.60	5554321000
5.80	5555432100
5.06	5555433100
4.60	5555543200
4.42	5555543343

TABLE XII

PARETIC FLUID "I"

Particle Size of Sols = 17.5 Millimicrons

pH OF SOL	TEST READING
7.35	3333100000
6.18	5554210000
5.77	5554210000
5.30	5555421000
4.56	5555541000
4.49	5555432310
4.10	5555555533
3.96	5555555554

The fact that pH and particle size exert this compensating effect is, perhaps, a part of the explanation for the fact that so many different techniques, as practiced in different laboratories, all yield gold sols suitable for use in the Lange test. Also, the fact that both pH and particle size are so very sensitive to very slight variations in technique and, particularly, to variations in the concentration and purity of reagents used will explain why sols of the exact quality required in the Lange test are so difficult to prepare.

There probably is an optimum combination of pH and particle size which will give an ideal Lange test gold sol. Just what this optimum combination may be is still a question, although the results obtained to date seem to indicate that sols of small particle size, with relatively low pH (about 6) are more satisfactory than sols of larger particles and higher pH.

The explanation of why the sensitivity of a gold sol is dependent upon particle size and pH would appear to be fairly simple. It is a well-known fact in colloid science that, other things being equal, larger particles are less stable than smaller particles. Hence, an increase in particle size should give a more sensitive sol. Gold sols are negatively charged, due, doubtless, to

adsorption of negative ions. Hence, an increase in the concentration of negative hydroxyl ions (increase in pH) should increase the stability of the sol.

Preliminary studies indicate that the Lange test readings in tabes, syphilis, encephalitis, spinal meningitis, tubercular meningitis and poliomyelitis are influenced by the pH and particle size of the gold sol in the same manner as are the readings in paresis. Detailed data on studies with these diseases will be reported in a later paper.

SUMMARY

The sensitivity of gold sols in the Lange spinal fluid test for paresis increases with an increase in particle size and decreases with an increase in pH.

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THE EFFECT OF FEVER THERAPY UPON CARBOHYDRATE METABOLISM*

MELVIN B. KIRSTEIN, M.D., AND LEON BROMBERG, M.D.
ST. LOUIS, MO.

IT IS known that infectious fevers cause moderate degrees of hyperglycemia. Simpson,[1] and Hench and Slocumb,[2] among others, have observed the same phenomenon in artificial hyperthermia. According to these observers, the hyperglycemia is due to blood concentration which occurs during fever therapy.

We have also noted hyperglycemia during fever therapy (see Table I), but we differ in the interpretation of the phenomenon and feel that its comprehension is of theoretical as well as of practical significance. We are ready to concur in the statement that loss of fluids during hyperthermia generally causes an increased concentration of blood constituents, such as nonprotein nitrogen (see Table I), urea, etc. However, to attribute the increase in blood sugar to concentration contradicts the physiology of the regulation of the glycemic level.

It is known that when the sugar content of the blood is augmented, e.g., by the intravenous injection of glucose, the resultant hyperglycemia is only of short duration, and the blood sugar soon returns to its original level. This is brought about by a regulatory mechanism in the liver which acts by changing the velocity relationship of a reversible reaction. This reversible enzymatic reaction consists of glycogen storage in one direction and glycogen

*From the Laboratory and Department of Fever Therapy, Jewish Hospital, St. Louis.

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breakdown in the opposite direction. The blood sugar level per se is the primary factor influencing the relationship between the velocities of these two opposite processes. Whenever the concentration of the blood sugar rises above the postabsorptive (fasting) level, sugar is rapidly withdrawn from circulation due to an increase of the reaction velocity in the direction of glycogen storage. When, for example, one injects 1.0 to 1.5 gm. of glucose per kg. body weight into the blood stream of a dog, the ensuing hyperglycemia disappears within thirty minutes.

The regulatory mechanism is exquisitely fine and responsive in its tendency to maintain a constant blood sugar level. This ready adaptability makes it possible for the blood sugar to remain almost immutable in the postabsorptive state. Although glucose is constantly withdrawn from the circulation by the peripheral tissues, the liver replaces it at the same rate that it is withdrawn. The rate of glycogenolysis is so astonishingly adaptable to the changing requirements that even during moderate physical exercise (when glucose is withdrawn from the blood stream at an increased rate) no demonstrable changes in the blood sugar level occur. Therefore, one is compelled to raise the question, why, if this fine regulatory mechanism so efficaciously disposes of hyperglycemias resulting from the injection of glucose, should it fail to take care of the relatively slight and gradual increases in glucose concentrations occurring during artificial fever. Thus all that is known concerning the mechanism which regulates the blood sugar level is in direct conflict with the assumption that hyperglycemia during fever therapy is due to blood concentration.

TABLE I

EFFECT OF ARTIFICIAL FEVER THERAPY UPON BLOOD SUGAR, NONPROTEIN NITROGEN AND PHOSPHORUS

Temperature Range—104-106 $\frac{8}{R}$. Duration of Fever 3-6 Hours.

Apparatus: Kettering Hypertherm

SUGAR MG. %		N.P.N. MG. %		PHOSPHORUS MG. %	
BEFORE $\frac{8}{R}$	AFTER $\frac{8}{R}$	BEFORE $\frac{8}{R}$	AFTER $\frac{8}{R}$	BEFORE $\frac{8}{R}$	AFTER $\frac{8}{R}$
84	92	27	33	---	---
89	93	19	22	---	---
87	109	--	--	---	---
88	94	26	30	2.9	2.1
84	87	28	38	2.7	1.9
97	98	24	27	2.5	2.2
80	101	16	21	---	---
88	93	--	--	---	---
83	95	15	14	---	---
95	100	20	18	---	---
92	105	20	22	---	---
83	115	--	--	3.4	1.5
86	87	--	--	3.2	1.5
69	85	16	19	2.6	1.3
81	93	20	23	3.6	2.4
86	93	--	--	3.1	2.7
87	89	18	30	4.8	4.8
84	88	20	17	3.5	2.9
82	82	16	16	3.8	3.7
80	85	17	24	4.0	3.8
80	85	15	20	4.1	3.8

Each horizontal line represents one patient.

An additional contradiction to the concentration theory is found in the fact that the inorganic blood phosphates show a substantial drop during hyperthermia. Our results (see Table I) show this fall in phosphorous and correspond to those reported by Bischoff,[3] Daly,[4] and others. The decrease in inorganic phosphates is readily explained by an increased rate of glucose utilization, due to a markedly increased metabolic rate during fever. Inorganic blood phosphates invariably decrease whenever increased utilization of glucose occurs (as after insulin administration, after the ingestion of glucose and during exercise). This adequately accounts for the drop in inorganic blood phosphates during fever therapy.

The increased rate of glucose utilization also explains the hyperglycemia during elevated body temperatures. Loseke and Gunderson[5] have reported marked depletion of liver glycogen in rabbits undergoing artificial fever. Somogyi[6] has emphasized in a recent paper that whenever blood sugar is utilized by body tissues at an increased rate (such as after insulin injection, during exercise and during hyperthermia), the glycogen reserve of the liver becomes depleted. This is due to the fact that the accelerated withdrawal of blood sugar by the peripheral tissues causes an increased rate of glycogen breakdown in the liver, so that the sugar content of the blood may be restored to its normal level. This compensatory process frequently overshoots its aim and hyperglycemia results. Individual differences in this regulatory mechanism explain the variations in the degree of the hyperglycemia encountered. In some instances the hyperglycemia is slight; in others the blood sugar rises as much as 20 to 30 mg. per cent above the fasting level. Therefore, we conclude that blood concentration is not the cause of the hyperglycemia during artificial fever.

This concept is of practical significance in fever therapy. Due to the increased combustion of sugar, fever tends to deplete the glycogen reserve of the body tissues, especially that of the liver. This depletion may become so excessive as to result in ketosis. Such an occurrence has been brought to our attention through a personal communication from Dr. H. W. Kendall, of the Kettering Institute, Dayton, Ohio. The depletion, even if it does not lead to ketosis, is certainly undesirable. Because of this, we feel that it is of considerable value to prepare patients for fever therapy by giving them liberal carbohydrate diets between sessions. In order to avoid a gain in weight to which these patients show a distinct tendency, we suggest a moderation of fats.

The preparation of diabetic persons for fever therapy is especially pertinent. One of the important pathologic factors in diabetes is the tendency toward an increased rate of glycogenolysis in the liver. As Somogyi has recently emphasized, if any factor which stimulates glycogenolysis (such as artificial fever) is superimposed upon this, the result may be excessive hyperglycemia, glycosuria, and frequently ketosis. (This may also explain the response of persons with diabetes to infectious fevers.) The vicious process can be combatted by means of adequate administration of carbohydrates and by inhibiting the rate of glycogenolysis with insulin. We feel that this concept should be applied to diabetic patients, when subjected to artificial fever.

CONCLUSION

Hyperglycemia during fever therapy is not due to blood concentration, but to disturbances in carbohydrate metabolism. This concept may have an important bearing upon the preparation of patients for fever therapy.

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IS THERE A PLATELET-REDUCING SUBSTANCE IN THE SPLEEN OF THROMBOCYTOPENIC PURPURA?*

RALPH H. MAJOR, M.D., AND C. J. WEBER, PH.D.
KANSAS CITY, KAN.

TROLAND and Lee[1] in January, 1938, described a platelet-reducing substance in the spleen of patients suffering from thrombocytopenic purpura. Later in July of the same year, they described their findings in detail and reported that extracts from three spleens from patients suffering from thrombocytopenic purpura contained a substance which produced a marked fall in the number of blood platelets in rabbits. They described falls as great as 605,000 per c.mm. to 100,000 per c.mm. in twenty-four hours. Not long after the first report we had the opportunity to study the spleens removed from two patients suffering from thrombocytopenic purpura. We delayed our report somewhat, hoping that additional cases would be obtained. Although we have seen some additional patients during the past year splenectomy was not recommended or carried out in any of them.

Patient M. G., female, aged 19 years, was admitted to the hospital February 1, 1938. She presented the typical history and findings of thrombocytopenic purpura. She had numerous petechiae over the legs and arms, her spleen was enlarged, and her blood platelet count varied from 85,000 to 50,000. The patient was operated upon February 22. The spleen was removed, and was found to measure 12 by 3.6 by 3 cm.; it weighed 170 gm., had a smooth surface, distinct notching, and no adhesions. Following the operation the patient's platelet count rose to 150,000 on February 25, and on March 5 it reached 350,000. The patient has had no further purpuric symptoms.

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*From the Hixon Laboratory of Medical Research, University of Kansas School of Medicine, Kansas City.

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The extract from the spleen was prepared in the manner described by Troland and Lee in their first article. The spleen was taken directly from the operating room and ground in a food chopper. Twice its volume by weight of acetone was added and the mixture allowed to stand in a sealed jar. After seventeen days the supernatant fluid was filtered off and collected, and the acetone evaporated off, leaving a thick brownish material. This material was dissolved in 125 c.c. of normal saline solution. Table I shows the effect of this solution on intravenous injection.

TABLE I

DATE	FIRST COUNT	SECOND COUNT	REMARKS
		Rabbit No. 1	
2/27/38	460,000	500,000	
3/ 2/38	470,000	480,000	
3/ 4/38	420,000	440,000	
3/ 9/38	500,000	440,000	10:00 A.M. 10 c.c. spleen extract intravenously
3/10/38	590,000	530,000	10:00 A.M.
	510,000	520,000	5:00 P.M.
3/11/38			8:30 A.M. 20 c.c. spleen extract intravenously
	780,000	750,000	1:30 P.M.
	630,000	580,000	5:30 P.M.
3/12/38	480,000	410,000	8:30 A.M.
		Rabbit No. 2	
3/18/38	500,000	480,000	
3/19/38	480,000	480,000	
3/21/38	560,000	510,000	11:15 A.M. 20 c.c. spleen extract intravenously
3/21/38	580,000	620,000	5:20 P.M.
3/22/38	400,000	440,000	5:00 P.M.
3/23/38	410,000	450,000	10:20 A.M.
		Rabbit No. 3	
3/18/38	440,000	500,000	
3/19/38	460,000	520,000	
3/21/38	510,000	540,000	11:15 A.M. 10 c.c. spleen extract intravenously
	550,000	550,000	5:20 P.M.
3/22/38	510,000	510,000	5:00 P.M.

The platelet counts were made by the method of Rees-Ecker. The results of these observations are not at all conclusive, the blood showing at times an increase, rather than a decrease, following injection.

The second patient, D. T., a girl 5 years of age, presented the typical history and clinical appearance of purpura hemorrhagica. The patient's platelet counts before operation varied from 80,000 to 90,000. The patient was operated upon May 18, 1938. The spleen weighed 89 gm. and measured 11 by 6.5 by 2 cm. in size. Following the operation the patient's count was 122,000 on May 22, and during the last week in the hospital it remained above 250,000.

The extract from the spleen of the second patient was prepared in a manner similar to that of the first patient. The ground-up spleen was allowed to remain in a sealed jar with the acetone for twenty-one days. As the spleen was smaller the brownish material was taken up in a smaller quantity of fluid, only 60 c.c. of salt solution being employed. The results of these tests are shown in Table II.

TABLE II

DATE	FIRST COUNT	SECOND COUNT	REMARKS
		Rabbit No. 2	
6/ 7/38	580,000	540,000	8:45 A.M. 20 c.c. spleen extract intravenously
	500,000	460,000	2:45 P.M.
6/ 8/38	540,000	520,000	8:45 A.M.
6/22/38	790,000	810,000	10:30 A.M. 20 c.c. spleen extract intravenously
	480,000	500,000	6:00 P.M.
6/23/38	380,000	350,000	10:30 A.M.
6/24/38	700,000	750,000	10:30 A.M.
		Rabbit No. 4	
6/22/38	730,000	700,000	10:30 A.M. 20 c.c. spleen extract intravenously
	560,000	520,000	6:00 P.M.
6/23/38	570,000	550,000	10:30 A.M.
6/24/38	600,000	650,000	10:30 A.M.

The splenic extract from this patient apparently produced a definite fall in platelet count. The figures, however, are not nearly so striking as those reported by Troland and Lee. In Table III are the platelet counts obtained in rabbits injected with normal spleen extract, extract from the spleen in Banti's disease, and extract from the spleen in myelogenous leucemia.

TABLE III

DATE	FIRST COUNT	SECOND COUNT	REMARKS
		Rabbit No. 1	
4/ 6/38	610,000	600,000	9:30 A.M. 20 c.c. normal spleen extract intravenously
	650,000	620,000	3:30 P.M.
4/ 8/38	570,000	540,000	9:30 A.M. 30 c.c. extract intravenously
	580,000	610,000	3:30 P.M.
		Rabbit No. 2. Banti's Disease	
5/ 9/38	610,000	580,000	
5/10/38	600,000	530,000	8:00 A.M. 10 c.c. spleen extract intravenously
	450,000	430,000	2:00 P.M.
5/11/38	550,000	530,000	8:30 A.M.
		Rabbit No. 1. Myelogenous Leucemia	
5/24/38	420,000	460,000	3:00 P.M.
5/25/38	420,000	450,000	10:00 A.M. 10 c.c. spleen extract intravenously
5/25/38	450,000	470,000	4:00 P.M.
5/26/38	480,000	490,000	10:00 A.M.
5/27/38	490,000	440,000	10:00 A.M.

COMMENTS

In the splenic extract from the first patient with thrombocytopenic purpura the extract in the rabbit produced an increase in the blood platelet count, while the spleen from the second patient produced a definite fall in the platelet count. Variations in both, however, were not striking and have been observed in the platelet counts of rabbits injected with a variety of materials. The divergence of these findings from those of Troland and Lee may be due to some difference in the method of extraction. In their first report they

stated that the supernatant fluid was poured off "after about two weeks," while the second report describes the periods as varying from six days to one hundred and two days.

Additional data would seem to be necessary before accepting as established, the presence of a platelet-reducing substance in the spleen of thrombocytopenic purpura.

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THE EFFECT OF GASTRIC MUCIN ON THE HEMOGLOBIN REGENERATION IN ANEMIC DOGS*

H. S. Wigodsky, M.S., R. A. Bussabarger, M.D.† and S. J. Fogelson, M.D.
Chicago, Ill.

THE work presented here was performed in an attempt to determine whether a commercial gastric mucin preparation stimulates blood formation in anemic dogs, through either the action of the mucin or some substance contained in the preparation. The work was prompted by the clinical observation that gastric mucin appeared to stimulate hemoglobin formation in "peptic" ulcer patients recovering from acute massive hemorrhage.

Whipple and his associates[1] in 1925 described a quantitative method for the determination of the ability of various substances to stimulate blood regeneration. During a period of more than a decade they have applied this method to innumerable substances.[2-10] Dragstedt and his co-workers[11, 12] have used it in an investigation of hemoglobin production in gastrectomized dogs. Riecker[13] has commented unfavorably on the errors of the method and the results obtained by Whipple. It was employed in the present study as the only available method for estimating "quantitatively" the ability of substances to promote hemoglobin formation.

METHOD

Eight dogs were used in this study. The procedure employed was essentially that of Whipple and his associates,[14] with slight modifications to be described below. The basal diet consisted of salmon bread to which were added the various supplements whose activities were to be determined. By means of frequent bleedings the dogs were maintained at a hemoglobin level of 5.50 to 6.50 gm. of hemoglobin per 100 c.c. of blood. When the animals

*From the Departments of Physiology and Pharmacology, and the Department of Surgery, Northwestern University Medical School, Chicago.
†Jessie Horton Koessler Fellow of the Institute of Medicine of Chicago.
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had been satisfactorily depleted of their stores of blood-forming materials as shown by a more or less constant rate of hemoglobin formation, control periods were established, ranging from four to seven weeks.

Series I: Following the control periods, 25 gm. of mucin suspended in water were added to the daily basal diet for a period of two weeks, followed by a two-week period during which they received only salmon bread. Since the mucin preparation was found to contain 1 mg. of iron per gram of mucin, the dogs were then given ferric ammonium citrate equivalent to 25 mg. of iron per day for a two-week period, followed by another two-week period during which the dogs received only salmon bread.

Series II: This group of 4 dogs was used to investigate the effect of feeding periods of longer duration. Following the control periods, 30 gm. of mucin suspended in water were added to the basal diet daily for four weeks. The animals were then fed only salmon bread for a period of two weeks. This was followed by a four-week period during which the animals received daily by stomach tube 30 c.c. of a 0.6 per cent ferric ammonium citrate solution, an amount of iron equivalent to the iron content of the mucin previously fed. A three-week "carry-over period" on salmon bread was allowed before the next supplement was added to the diet. During the next supplemental period the dogs received daily for four weeks 250 gm. of fresh beef liver ground together with 340 gm. of salmon bread. This was followed by a four-week "carry-over period."

CALCULATION

Blood studies and blood volume determinations were made in essentially the same manner as that of Whipple and his collaborators. However, the amount of hemoglobin produced was calculated by a method different from that used by Whipple, as well as by the Whipple method.

The calculation of the amount of hemoglobin formed during a given period requires the consideration of two factors: (a) the amount of hemoglobin withdrawn by bleeding during the period; (b) changes in the total quantity of hemoglobin in the body during this period. The first is measured directly; the second must be calculated. Whipple's method for calculation of the hemoglobin formed may be represented by the following formula:

$$\text{Hb formed} = \text{Hb withdrawn} + (\text{per cent } Hb_2 - \text{per cent } Hb_1),$$

where Hb_1 and Hb_2 are the blood hemoglobin percentages at the beginning and at the end of the period, respectively. This method arbitrarily assumes that each unit change in percentage of blood hemoglobin represents a change of 1 gm. in the total circulating hemoglobin. The assumption is valid only when the blood volume remains constant at 725 c.c. (assuming 100 per cent hemoglobin = 13.8 gm. per 100 c.c.).

The method of calculation devised by us may be represented by the following formula:

$$\text{Hb formed} = \text{Hb withdrawn} + (Hb_2 - Hb_1),$$

where Hb_1 and Hb_2 represent the total circulating hemoglobin in grams at the beginning and end of the period, respectively. The accuracy of Whipple's

TABLE I

PARTIAL PROTOCOL SERIES II

DOG NO. 20

Key to Columns

1. Days anemic
2. Weight in kilograms
3. Millions R. B. C. per c.mm.
4. Grams hemoglobin per 100 c.c.
5. Hematocrit mm.
6. Amount of blood withdrawn, c.c.
7. Centrifuge tube readings—control
8. Centrifuge tube readings—dye
9. Reading on colorimeter
10. Plasma volume
11. Blood volume
12. Total circulating hemoglobin
13. Amount of hemoglobin withdrawn, gm.
14. Total amount of hemoglobin withdrawn, gm.
15. Correction
16. Hemoglobin produced

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
62	12.8	4.27	6.00	20.0	76	2.1/10.7	1.5/ 8.8	0.777	789	1042	62.4	4.56	20.58	-23.10	-2.52
65			6.53		105							5.17			
67			6.26		50							3.13			
69	12.5	3.48	5.38	19.0	20	2.1/11.5	1.9/11.2	0.790	795	1022	55.0	1.08	12.86	- 7.4	5.46
73			6.41												
74			5.74												
76	12.5	4.27	5.65	19.0	16	1.9/ 9.6	1.9/ 9.6	0.770	768	1023	57.8	0.90	1.08	+ 2.8	3.88
80			5.85												
83	12.7	4.73	6.83	20.0	17	2.1/11.0	2.2/11.0	0.750	823	1087	74.2	1.16	0.90	+16.4	17.30
86			6.49		55							3.57			

TABLE II

AVERAGE HEMOGLOBIN PRODUCTION PER WEEK

ANIMAL	B.H.P.*	MUCIN		IRON		LIVER	
		FED	CARRY-OVER	FED	CARRY-OVER	FED	CARRY-OVER
A. Our Method of Calculation							
Series I† 1	5.83	18.02	8.00	9.62	9.86		
Series I 2	6.53	24.97	9.91	14.63	4.84		
Series I 3	14.35	24.97	6.66	23.70	8.90		
Series I 4	15.09	21.79	15.53	15.83	6.35		
Series I Avg.	10.46±1.68	22.4±1.10	10.03	15.45±1.98	7.49		
Series II† 12	3.33	2.51	8.09	5.52	5.37	11.95	9.98
Series II 17	4.22	10.42	6.43	5.73	6.59	13.74	14.72
Series II 18	6.88	12.88	8.38	10.92	5.76	21.04	15.49
Series II 20	8.21	20.31	8.20	7.67	5.69	8.92	17.55
Series II Avg.	5.66±0.76	11.53±2.47	7.77	7.46±0.85	5.85	13.91±1.74	14.43
Average Series I and II	8.06±1.05	16.99±1.87	8.90	11.70±1.47	6.67		
B. Whipple's Method of Calculation							
Series I† 1	12.53	13.57	8.20	12.72	5.71		
Series I 2	8.54	26.60	10.24	13.33	6.54		
Series I 3	13.65	30.27	7.81	22.40	8.65		
Series I 4	15.75	23.79	15.68	16.64	5.30		
Series I Avg.	12.62±1.02	23.56±2.91	10.48	16.27±1.50	6.55		
Series II† 12	2.80	4.91	5.69	5.98	4.20	9.58	10.78
Series II 17	4.49	6.70	3.98	9.83	6.93	9.37	14.07
Series II 18	5.06	11.83	5.53	10.17	7.26	17.72	16.69
Series II 20	7.64	22.78	4.70	7.50	7.26	7.63	17.35
Series II Avg.	5.00±0.68	11.56±2.72	4.98	8.37±0.67	6.41	11.08±1.52	14.72
Average Series I and II	8.80±1.41	17.55±2.87	7.72	12.32±1.26	6.48		

*Basal hemoglobin production.
†For details see Methods.

method is limited by the introduction of an arbitrary constant; the accuracy of our method is limited by the accuracy of the blood volume determinations.

RESULTS

Table I shows a portion of a typical protocol of one of the animals used in this study. Table II shows the average weekly hemoglobin production of each of the animals during each of the experimental periods, together with the averages and probable errors. Our method was used to calculate Table IIA. Whipple's method was used to calculate Table IIB. Table III is a condensed protocol of one of the animals of the series.

The average basal hemoglobin production for all of the dogs when calculated by the Whipple method was 8.80 ± 1.41 gm. per week. The addition of mucin to the diet increased the hemoglobin production to 17.55 ± 2.87 gm. per week, or an increase of nearly 100 per cent, which, however, is not significant statistically. The addition of iron increased the hemoglobin production by 40 per cent. In each case the values are not statistically significant.

When our method (use of the blood volume determination) of calculation is employed, practically the same average values are obtained, but indi-

TABLE III

CONDENSED PROTOCOL

DOG NO. 20

DAYS ANEMIC	PERIOD	WEIGHT	HB.	BLOOD VOLUME	TOTAL CIRCULATING HB.	TOTAL HB. WITHDRAWN	HB. PRODUCED PER WEEK
62	Control	12.8	6.00	1042	62.4		
68	Control	12.5	5.38	1022	55.0	12.86	5.46
76	Control	12.5	5.65	1023	57.8	1.08	3.88
83	Control	12.7	6.83	1087	74.2	0.90	17.30
97	Control	11.6	5.50	982	54.0	19.47	-0.73*
104	Control	11.6	6.53	1083	70.8	6.54	23.34
111	Mucin	10.9	6.53	1018	66.4	12.91	8.51
118	Mucin	11.3	7.45	1018	75.7	1.11	10.41
125	Mucin	11.3	7.79	853	67.2	50.56	42.06
132	Mucin	10.8	6.00	946	56.7	30.25	20.25
146	Carry-over	10.9	6.49	964	62.6	5.9	16.40*
153	Iron	11.1	6.57	1004	65.9	1.23	4.53
160	Iron	11.0	6.97	819	57.1	8.23	-0.55
174	Iron	10.4	6.87	962	66.1	17.70	26.70*
181	Carry-over	10.5	6.30	986	62.1	7.82	3.82
188	Carry-over	9.9	6.62	942	62.3	4.78	4.98
195	Carry-over	9.9	6.85	893	61.2	9.38	8.28
202	Liver	11.1	6.15	1059	65.1	1.23	5.13
209	Liver	11.8	6.40	1620	103.6	9.15	47.65
216	Liver	12.0	5.90	1120	66.1	16.10	-21.40
223	Liver	12.5	7.20	963	69.2	1.24	4.34
230	Carry-over	12.3	7.20	1111	80.0	20.05	30.85
237	Carry-over	12.2	7.31	1062	77.6	12.56	10.16
244	Carry-over	12.7	6.53	1158	75.7	27.20	25.30
251	Carry-over	12.4	7.00	1000	72.0	9.58	3.88

*Two weeks hemoglobin production.

vidual variations are reduced to a point where the addition of the various supplements to the diet resulted in statistically significant increases in hemoglobin production, except in the case of iron.

DISCUSSION

Before discussing the significance of the results obtained in this study, the reliability of the method employed to increase the rate of hemoglobin production must be considered. Examination of Table II reveals a wide variation in hemoglobin production from week to week. This variation is greatest when Whipple's method of calculation is used, a fact which is revealed by the greater probable error of the averages. However, even with our method of calculation the variations are great. These variations are inherent in the method, for examination of Whipple's data reveals equally great discrepancies. It has been impossible for us to duplicate *quantitatively* Whipple's results with iron and liver; *qualitatively* his results are confirmed. One can only conclude that under the condition of the method, the rate of hemoglobin formation is not constant and uniform when the same supplements are fed. In view of this fact *the method cannot be truly quantitative, and its results are acceptable only when the data are sufficiently extensive to permit a statistical analysis.*

The results show that mucin definitely promotes the formation of hemoglobin in the dog. Since the administration of an amount of iron equivalent

to that contained in the mucin had one-half the effect of the mucin, the activity of the latter cannot be attributed solely to its iron content. Whether the additional effect is due to a more adequate protein intake, or to some specific hematopoietic agent contained in commercial mucin, we are unable to say. These findings, however, probably explain the clinical impression that patients on mucin therapy rapidly regenerate hemoglobin.

SUMMARY AND CONCLUSIONS

1. In each of two series of 4 anemic dogs, a commercial gastric mucin preparation stimulated hemoglobin production 55 to 70 per cent more than could be accounted for by the amount of iron present in the preparation. However, the stimulus was not as great as when beef liver was fed.

2. A modification of Whipple's method for calculation of the results in these types of experiments has been introduced and probable errors in Whipple's calculations have been pointed out.

3. Notwithstanding our modification of the Whipple method for determining the effect of various substances on hemoglobin regeneration in anemic dogs, we believe that the inherent errors of this method make it a qualitative rather than a quantitative one, unless a sufficient amount of data are obtained to permit statistical analysis.

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THE EFFECT OF SODIUM BROMIDE ON THE NUTRITION AND THE GASTROINTESTINAL TRACT OF EPILEPTIC PATIENTS*

ALEX J. ARIEFF, M.D., CHICAGO, ILL.

BECAUSE of the extensive and useful administration of the bromides in epilepsy, an inquiry has been made into some possible untoward action. Among the common complaints and observations attributed to bromide intoxication are skin eruptions, drowsiness, upper respiratory infections, and, at times, anorexia and weight loss.

Lately Paskind[1] reported on observations of a large number of extramural epileptic patients who were treated with sodium bromide over a period of many years and showed that bromides do not cause mental deterioration, another observation sometimes attributed to the use of bromides.

In the clinic for epilepsy at the Northwestern University Medical School, bromides are found to be the most efficacious drugs in the treatment of the convulsive state. By regulating the individual dosages in accordance with clinical observations and frequent blood bromide determinations (Wuth method[2]), very few toxic complications have resulted.

The present study concerns itself with nutrition, function of the gastrointestinal tract, and metabolism.

Bromides, when taken orally, are rapidly absorbed from the gastrointestinal tract and also secreted by it, replacing the chloride ion (Nencki and Schoumow Simanowsky,[3] Bolgar[7]). Replacement of the chloride ion occurs in all tissues, including the blood (Boshes,[4] Mason[5]). When injected, it is rapidly secreted but slowly excreted from the body. Bromides are found normally in the body tissues in very small quantities (less than a fraction of a milligram) which vary with the diet, but they are not necessary to normal body metabolism (Winnek and Smith[11]).

Since the bromide ion is secreted by the stomach with the chloride ion, hydrobromic acid must be present with hydrochloric acid in the stomach secretion. The effect of hydrobromic acid on peptic digestion in in vitro experiments was therefore studied.

To determine peptic activity of hydrobromic acid, Mett's tubes, 20 mm. long filled with coagulated egg albumen,[6] were placed in different mixtures as seen in Tables I and II. It can be seen that the greater the concentration of bromide in the acid, the less digestion occurred, although the peptic digestion in all experiments reached a degree which is considered normal. One could then say that hydrochloric acid alone is a faster activator of pepsin, although the retarding action of hydrobromic acid clinically should not be apparent.

*From the Department of Nervous and Mental Diseases, Northwestern University Medical School.

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TABLE I

EFFECT OF HYDROBROMIC ACID ON PEPTIC DIGESTION

EXPERIMENTS	PEPSIN SOLUTION	0.18% HCl	0.18% HBr	DIGESTION IN 20 MM. METT'S TUBES		PER CENT AVERAGE DIGESTION 24 HOURS
				1	2	
1	1 c.c.	15 c.c.	0	20/20	20/20	100
2	1 c.c.	0	15 c.c.	11/20	14/20	60
3	1 c.c.	7.5 c.c.	7.5 c.c.	20/20	16/20	90
4	1 c.c.	0	0	0/20	0/20	0
		15 c.c. H_2O				
5	1 c.c.	10 c.c.	5 c.c.	20/20	20/20	100

TABLE II

EXPERIMENTS	PEPSIN SOLUTION	0.2% HCl N/20	0.2% HBr N/20	DIGESTION IN 20 MM. METT'S TUBES			PER CENT AVERAGE DIGESTION 17 HOURS
				1	2	3	
1	1 c.c.	15 c.c.	0	20/20	18/20	18/20	95.0
2	1 c.c.	10 c.c.	5 c.c.	19/20	11/20	13/20	71.5
3	1 c.c.	7.5 c.c.	7.5 c.c.	16/20	13/20	14/20	71.5
4	1 c.c.	5 c.c.	10 c.c.	13/20	10/20	10/20	55.0
5	1 c.c.	0	15 c.c.	7/20	7/20	8/20	36.5

Table III indicates similar in vitro experiments to study the effect of bromide salts on tryptic digestion. Sodium bromide had no retarding effect on the tryptic digestion of casein.

TABLE III

EFFECT OF HALIDE SALTS ON TRYPTIC DIGESTION

EXPERIMENTS	CASEIN	TRYPSIN	HALIDE SALT	DIGESTION AFTER 15 MINUTES
1	10 c.c.	1.4 c.c.	None	Complete
2	10 c.c.	1.4 c.c.	1.0 c.c. 10% NaCl	Complete
3	10 c.c.	1.4 c.c.	1.0 c.c. 10% NaBr	Complete
4	10 c.c.	1.4 c.c.	0.5 c.c. 10% NaCl 0.5 c.c. 10% NaBr	Complete
5	10 c.c.	1.4 c.c.	2.0 c.c. 10% NaBr	Complete

Epstein[8] felt that in clinical experiments in three cases, he demonstrated an increased acidity and an increase in peptic digestion by bromide medication. Since hydrobromic acid is a stronger acid than hydrochloric acid, one would expect a decrease in pH, but the slight difference would not be clinically significant.

Bromides have been recommended by many workers in functional gastrointestinal disturbances, but their actual value never has been determined. Baltaceanu[9] reported no constant action of bromides with or without atropine on the gastric acidity.

As previously stated, from time to time epileptic patients on bromide therapy appear to lose weight. In some anorexia is a complaint. With this in mind, a group of 34 patients, who, with the exception of one, had been followed from one to six years, was studied with regard to nutrition, gastro-

intestinal complaints, weight, gastric analysis, blood bromide, urine, stool and blood examinations. Eight of these patients suffered from an organic epilepsy and 26 from the idiopathic type.

The age distribution of the patients studied was as follows: one patient was under 10 years; 7 were from 10 to 20 years; 11, from 20 to 30 years; 7, from 30 to 40 years; 6, from 40 to 50 years; and 2, from 50 to 60 years.

The results of urine, blood, and stool examinations revealed nothing abnormal. Urines occasionally showed a trace of albumin, which usually disappeared on subsequent examinations. The results of microscopic examinations showed nothing abnormal.

Blood examinations included red and white blood cell counts and differential count and hemoglobin determinations. Mild secondary anemias were occasionally discovered even before the institution of bromide therapy, but such a condition is not unusual in clinic patients who are on an inadequate diet. This condition responded well to an antianemic regime. The results of stool examinations were all within normal limits, with the exception of positive benzidine reactions; patients with these reactions were not on meat-free diets at the time.

All patients were questioned as to the presence of gastrointestinal complaints. Many were constipated and were relieved by placing the bromide in a mixture of infusion of *Adonis vernalis*. In some cases it was necessary to give mineral oil or mild laxatives with modifications of their diet. Where gastrointestinal complaints were made in a few cases, they had been present before bromide therapy. In no case did patients have gastric disturbances directly attributable to the bromide. Most of the patients with such complaints felt better on bromides. Anorexia was not an uncommon complaint, but when present was observed in those patients who became toxic or who ran a high blood bromide (of 300+ mg. per cent). In these patients, when the blood bromide level was decreased and their diet increased, there was no loss of weight.

In Table IV one can correlate length of time patients were on sodium bromide with their weight, before and after starting medication. The state of their nutrition and average blood bromide are given.

One can see the variation in weight is within normal limits. In patient F. M. the weight loss was 18 pounds over a period of three to four years. Most of this weight loss was due to an inadequate caloric diet at the beginning of treatment. In the last year his weight has been stationary. In 5 patients, W. M., C. N., F. K., E. S., and F. M., all in the adolescent age, weight increased during their growth period in spite of their continued bromide intake. There was no correlation between duration of bromide therapy and weight gain or loss. The nutritional state was good in most of the cases and fair in only a few cases. There were no cases of malnutrition due to bromides.

The average blood bromide level attained in the clinic is usually around 200 mg. per 100 c.c. blood. With this level we see very few toxic cases, contrary to the statement of many workers that a blood bromide over 150 is toxic and dangerous.

TABLE IV

YEARS ON NaBr	NUTRITION	WEIGHT			AVERAGE BLOOD BROMIDE
		BEFORE	AFTER	DIFFERENCE	
1-2					
1. P. K.	Good	195	205	+10	225
2. E. T.	Good	182	172	-10	250
3. E. A.	Good	197	197	0	225
4. K. K.	Good	127	127	0	250
5. W. T.	Good	124	116	- 8	160
6. E. E.	Good	120	124	+ 4	250
7. N. J.	Good	130	138	+ 8	225
8. L. L.	Fair	98	94	- 4	150
9. M. P.	Fair	99	99	0	210
10. B. O.	Good	81	86	+ 5	225
11. W. M.	Good	49	66	+17	175
12. W. K.	Good	145	147	+ 2	200
2-3					
1. M. H.	Good	92	90	- 2	170
2. C. K.	Good	172	167	- 5	175
3. E. C.	Good	157	139	-18	225
4. E. R.	Fair	132	128	- 4	160
5. B. H.	Good	125	127	+ 2	160
6. M. L.	Good	120	109	-11	225
7. M. M.	Good	130	135	+ 5	175
8. C. N.	Fair	108	131	+23	125
9. L. J.	Good	138	131	- 7	175
3-4					
1. V. L.	Good	134	130	- 4	180
2. F. K.	Good	96	130	+34	175
3. C. L.	Good	193	187	- 6	190
4. I. W.	Fair	118	120	+ 2	250
4-5					
1. J. K.	Good	154	149	- 5	200
2. F. M.	Good	148	130	-18	175
3. K. R.	Fair	103	103	0	250
4. C. L.	Good	153	154	+ 1	200
6-8					
1. E. S.	Fair-Good	67	95	+28	250
2. A. J.	Good	127	128	+ 1	175
3. F. F.	Good	101	150	+49	150
4. C. R.	Good	136	136	0	200

One can reiterate that, when weight loss occurs in patients on bromides, the diet has been decreased because of anorexia, and when the diet is increased or supplemented by frequent feedings, weight can be restored.

Gastric analyses were done in 20 patients (Table V). Free acid varied from 8 to 49 and total acid varied from 24 to 65 units of N/10 hydrochloric acid. There were no patients with achylia gastrica as reported by Felsen,[10] or high acidity as reported by Dattner.[14] The total halides varied between 400 and 780 mg. per cent and the bromides from 25 to 50 per cent of the total halides. There was no relationship between change in weight and results in gastric analysis.

Because bromine is one of the halogen family, one might expect it to have an action similar to iodine. The possible action of bromide on the thyroid was studied. Margolin[12] and Hamilton[13] fed bromides to guinea pigs and albino rats and produced histologic changes in the thyroid gland similar to that produced by iodine, but not to such a marked degree. With this in mind, basal metabolic rates were determined on 13 patients when their blood

TABLE V

GASTRIC ANALYSES

	AVERAGE BLOOD BROMIDE	ACIDITY		TOTAL HALIDES	BROMIDE	PER CENT Br/HALIDES
		FREE	TOTAL			
1. P. K.	225	49.0	65.0	630	250	40
2. V. S.	180	19.0	33.0	400	200	50
3. F. K.	175	22.0	40.0			
4. J. K.	200	37.0	62.0	450	200	44
5. E. C.	225			720	300	41
6. F. M.	175	47.5	60.5			
7. K. K.	250	22.0	36.0			
8. F. F.	150			780	260	33
9. W. T.	160	17.5	27.8	560	150	27
10. E. E.	250	+		680	250	36
11. E. H.	200	18.0	24.0	780	250	32
12. B. H.	160	19.5	27.3	620	280	45
13. C. R.	200	9.4			280	
14. M. L.	225			430	215	50
15. N. J.	225	8.0	36.0	480	125	26
16. L. L.	150	30.0	44.0			
17. M. P.	210	29.5	47.0			
18. E. S.	250	24.0	49.5			
19. C. S.	200	18.0	31.0			
20. I. W.	250	22.0	32.0			

bromide was at a high level. The rates were all within normal limits. None were over +20 or less than –20. In three cases the rates were –17, –16, and –20, but these rates could not be correlated with any other hypothyroid manifestation. One patient was toxic and ran a blood bromide over 300, but her basal metabolic rate was +7.8 per cent.

Sodium bromide is safe to use over a long period of time, but patients need to be observed for its possible toxic effects. Nutrition need not be affected. Weight can be kept at normal level with an adequate caloric intake.

CONCLUSIONS

1. Bromides are secreted by the stomach, displacing the chloride ions as in the blood and other tissues. Hydrobromic acid in vitro slightly retards peptic digestion but not enough to produce clinical symptoms.

2. Bromides have no retarding effect on tryptic digestion in vitro.

3. A high blood bromide has no effect on nutrition. Weight loss is produced only when there is anorexia or bromide toxicosis producing an inadequate caloric intake.

4. Gastric analyses of patients on bromides showed nothing significant. The chloride ion was displaced by bromide from 25 to 50 per cent.

5. A high blood bromide level produced no abnormal change in the basal metabolic rate.

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THE CORRELATION OF CLINICAL, ELECTROCARDIOGRAPHIC, AND CIRCULATION TIME FINDINGS IN DETERMINING THE CARDIAC STATUS IN INFECTIOUS DISEASES*

LOUIS H. SIGLER, M.D., PHILIP I. NASH, M.D., ISIDORE STEIN, M.D., AND SAMUEL EPSTEIN, M.D., BROOKLYN, N. Y.

THE question as to how frequently the heart is damaged during the course of infectious disease and how much such damage contributes to the symptomatology has not as yet been definitely answered. That pathologic changes of an acute nature are found on post-mortem examination in individuals who die from infectious disease has repeatedly been demonstrated in the past. This is true, however, in those who die and may not be true in survivors. Furthermore, even of those who die only a comparatively small number show pathologic changes. Thus Stone[1] found that of 259 autopsies of patients with lobar pneumonia, bronchopneumonia, and pneumococcus sepsis, 57 per cent of the first, 66 per cent of the second, and 38 per cent of the last had normal hearts.

An attempt has been made in the past few years to answer this question by electrocardiographic means by Brow,[2] Chagras,[3] Master and Romanoff,[4] Master, Romanoff, and Jaffee,[5] Degraff, Travell, and Yager,[6] Arrett and Harris,[7] Porter and Bloom[8] and others. More recently, studies of the circulation time have been used for the same purpose on 75 patients with lobar pneumonia by Hitzig, King, Bullowa, and Fishberg.[9] A few cases in which such study was made are also mentioned by Hitzig,[10] and by Tarr, Oppenheimer, and Sager.[11]

*From the Department of Medicine, Coney Island Hospital, Brooklyn.
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No correlation has, however, been made between the clinical manifestations, the electrocardiographic changes, and the circulation time, so as to attempt to arrive at a more exact estimation of the cardiac status.

This paper is an attempt at such correlation. It covers 81 patients with infectious disease on whom the circulation time was determined at the same time that electrocardiographic studies were made, and the findings were correlated with the subjective symptoms. It adds that many more cases of circulation time studies in infectious disease to the comparatively small number recorded in the literature.

MATERIAL AND METHODS EMPLOYED

Our series consisted of 69 patients with lobar pneumonia, 3 with meningococcus meningitis, 4 with upper respiratory infection, including one with Vincent's angina, 3 with influenza, one with ulcerative colitis, and one with empyema of the gall bladder. There were 39 females and 42 males, varying in age between 14 and 69 years. Twelve patients, all belonging to the pneumonia group, died, and 69 recovered. Only one case had come to autopsy.

The circulation time was determined by the saccharine and ether methods at the height of the disease or when symptoms were marked. In most cases where the saccharine time was normal, no ether time determination was done. Electrocardiograms were obtained at the same time as the circulation time and were repeated at intervals of one to four days. If the circulation time was abnormal in any case, a follow-up was made. Abnormalities in heart sounds (if any), the presence or absence of murmurs, cyanosis, and objective dyspnea were recorded at the same time.

Noteworthy changes in the electrocardiogram were artificially divided into first, second, and third degrees. In the first degree were placed such changes as notching or slurring of the QRS complexes in Lead I or Lead III where previously these complexes were normal: low voltage T waves in all leads and change from positive to negative T wave in Lead III. In the second degree were included low voltage QRS complexes in all leads with slight slurring or notching; isoelectric T wave in the Lead II and negative in Lead III. In the third degree were cases showing marked notching or slurring in the QRS complex with or without low voltage, and marked changes in the T wave in Leads I and II or all leads.

The limits of normality for the circulation time were set at 9 to 16 seconds for saccharine time, and 3.5 to 8 seconds for ether time. The circulation time was obtained only in 77 patients on whom 99 saccharine time and 28 ether time determinations were done. One hundred and ninety-three electrocardiograms were done on 80 patients at varying intervals. There were 4 patients on whom no circulation time but repeated electrocardiograms were obtained, and one patient on whom no electrocardiogram was obtained but circulation time was determined.

The objective clinical signs looked for were visible dyspnea, cyanosis, cardiac murmurs, or gallop rhythm, and changes in the heart sounds, such as diminished intensity, splitting, accentuation, or reduplication.

OBSERVATIONS

The Electrocardiogram.—Insignificant changes, such as slight variation in voltage of the QRS complex and of the T wave, and negative, isoelectric or diphasic P wave in Lead III. were frequently observed, but only 15 persons showed noteworthy changes that could be grouped under the first, second, and third degrees previously described. Of these the following changes were noted in the course of the disease: 2 changed from normal to first degree; 2 from normal to third degree; one from first to second; one from first to second then to normal; one from second to third degree; one from second to third degree then to normal; 4 from third degree to normal; one from third to first then to second degree; 3 from third to first degree; 2 from first degree to normal; one from second to first; and one from second to third and then first degree. All these changes occurred at various periods during the illness or in convalescence. One patient presented sinoauricular standstill with ventricular escape and ectopic auricular impulses on the third day of illness from meningitis which persisted till recovery.

The most frequent changes observed were in the direction of the T waves in Leads II and III. Only one case of prolonged P-R conduction time to 0.24 second was seen. Brow observed this condition in 14 out of 65 cases of typhoid fever. Master and his co-workers observed it in 35 per cent of cases of pneumonia. Our incidence is about the same as that of Arrett and Harris who found the condition in 2 out of 77 pneumonia patients. At no time did we observe changes in the R-T or S-T segment characteristic of early coronary occlusion reported by Master and his co-workers. An occasional slight depression or elevation and rounding was seen, but it was not characteristic of occlusion. Sometimes an auricular premature contraction was seen and sinus bradycardia and arrhythmia were frequent findings in convalescence. A few representative electrocardiograms are shown in Figs. 1 to 4.

It was found occasionally that improvement in the appearance of the T wave occurred when the clinical condition became definitely worse and even shortly before death. This is seen in Fig. 1 where all T waves became positive one day before death when they were almost isoelectric before. Post-mortem examination showed neither gross nor microscopic pathology of the heart. There was merely slight arteriosclerosis of the descending thoracic and abdominal portions of the aorta. In some cases the return to a normal T wave went hand in hand with the improvement of the patient's condition, as seen in Fig. 2.

The Circulation Time.—In 58 patients the saccharine time was normal and in 19 it was prolonged. Ether time was normal in 13 and prolonged in 7. Five patients showed prolonged saccharine time and normal ether time; two of these died and 3 recovered. In 3 patients the saccharine time was 8.5, 8, and 1.5 seconds, respectively, the last of which is far less than the minimum normal. Four patients had unusually prolonged saccharine time of 4, 3.5, 5 minutes, and 3 minutes 14 seconds, respectively, the first of which had also a prolonged ether time to 36 seconds. It is questionable, however, if the figures actually represent the circulation time. All 4 patients were stuporous at the time of

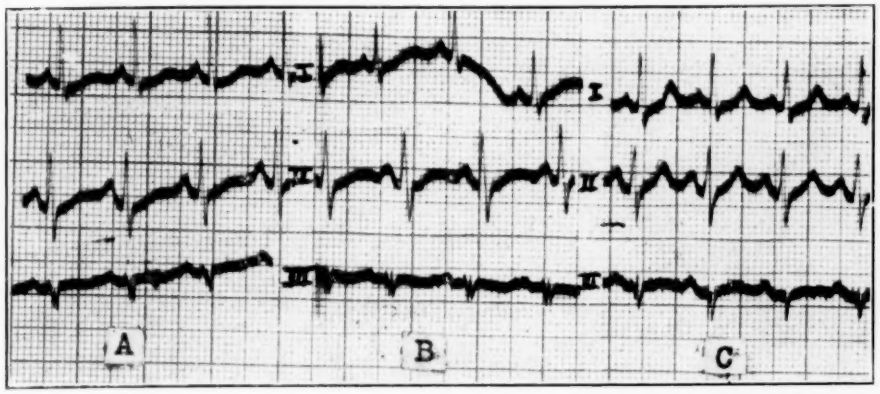

Fig. 1.—From a case of lobar pneumonia, type III, female 40 years old. *A*, Second day of illness. Rate 136. Tendency toward left axis deviation, slight depression of S-T segment, and very low voltage T wave in Leads I and II. Negative T wave in Lead III. *B*, Third day of illness. Rate 125. QRS complexes in Leads I and III are of lower voltage. *C*, Fifth day of illness and one day before death. Rate 136. T wave in all leads is positive.

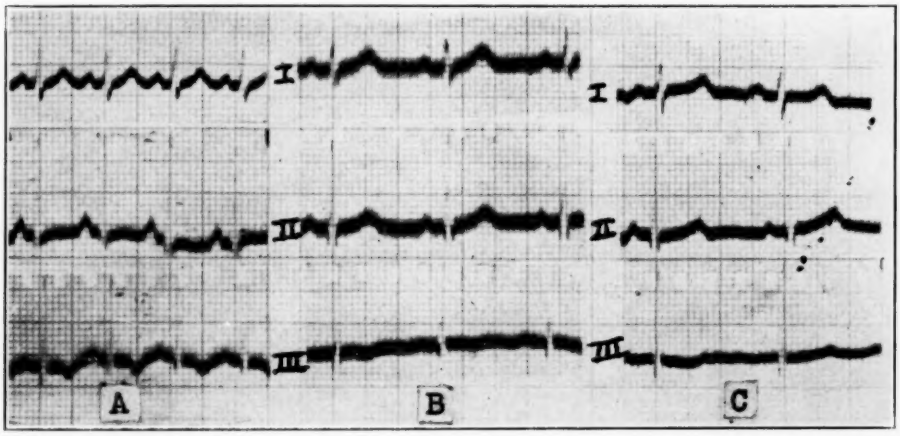

Fig. 2.—From a case of lobar pneumonia, type I, female 14 years old. *A*, Second day of illness. Rate 150. Isoelectric T wave in Lead II and negative T wave in Lead III. *B*, Fifth day of illness. Rate 85. T wave in Lead II positive and in Lead III still slightly negative. *C*, Eighth day of illness, beginning convalescence. Rate 83. T wave in all leads is now positive.

the test, and it is likely that the perception apparatus was at fault. Three of these recovered and one died. The average ether time in the entire series was 7.5 seconds, and the saccharine time was 13.3 seconds The average high saccharine time was 19 seconds.

Repeated circulation time determinations in 15 patients showed changes from time to time, as seen in Table I. Seven had a prolonged time early which returned to normal later. One of these died. In 2 patients the circulation time became faster and in 2 others it became somewhat prolonged but still within normal limits. No constant relationship was noted between the height of temperature and the rapidity of circulation time, as is shown in Table I.

The circulation time was prolonged more often among those patients who died than among those who survived, as shown in Table II. It was also found to be more frequently prolonged after 45 years of age, as shown in Table III.

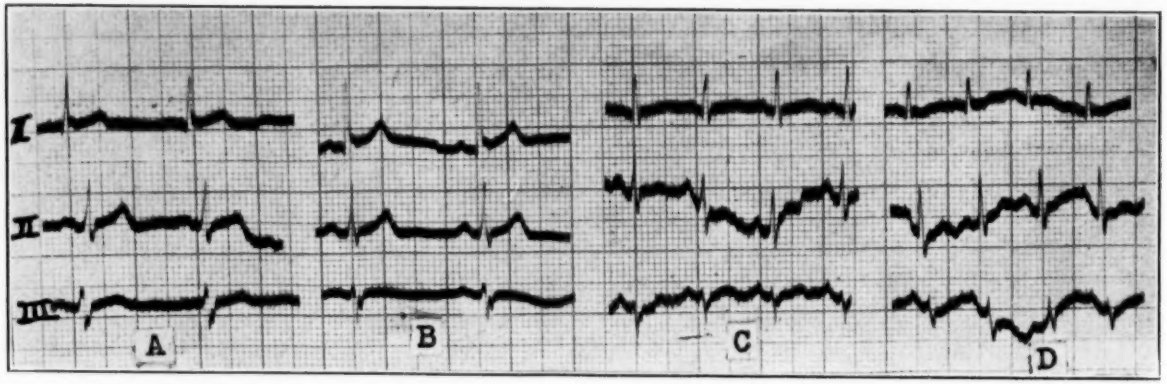

Fig. 3.—From a case of pneumonia, followed by empyema, male 30 years old. *A*, Third day of illness. Rate 76. Slight elevation and rounding of R-T segment in Lead I; tendency toward left axis deviation; T wave in all leads positive. *B*, Fifth day of illness. Rate 71. The R and T waves in Lead I of higher voltage with greater elevation and rounding of the R-T segment. *C*, Seventeenth day of illness. Rate 136. The QRS complex is of lower voltage and the T wave in Leads I and II approach isoelectric level, and in Lead III it is of somewhat higher voltage than previously. *D*, Nineteenth day of illness and one day before death. Rate 166. QRS complex in Lead I is of low voltage.

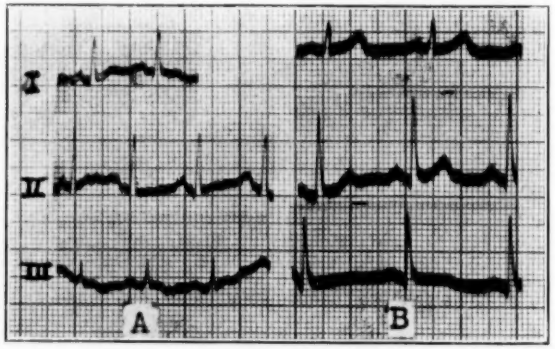

Fig. 4.—From a case of lobar pneumonia, female 26 years old. *A*, Third day of illness. Rate 136. Low voltage T wave in Lead I, tendency toward negative T wave in Lead II, and negative T wave in Lead III, with some coving of the R-T segment. *B*, Tenth day after onset of illness, during convalescence. Rate 79. The QRS complexes are of higher voltage in Leads II and III. The T wave is positive in Leads I and II, and isoelectric with positive tendency in Lead III.

TABLE I

SUMMARY OF CHANGES NOTED IN THE SACCHARINE TIME IN 15 PATIENTS

CASE NO.	DISEASE AND TERMINATION	DAY OF ILLNESS	SACCHARINE TIME	TEMPERATURE
1	Pneumonia type V	5th	11.4 sec.	104.8
	Recovered	8th	9.15 sec.	104.2
2	Pneumonia type XV	4th	24.0 sec.	102.0
	Recovered	5th	16.2 sec.	103.0
		12th	12.4 sec.	101.0
3	Pneumonia type I	5th	16.7 sec.	103.0
		11th	10.0 sec.	102.0
4	Pneumonia type II	6th	15.2 sec.	103.0
	Recovered	10th	13.5 sec.	100.0
		12th	14.6 sec.	100.0
5	Pneumonia type VII	11th	12.2 sec.	103.4
	Recovered	18th	16.2 sec.	98.6
6	Pneumonia type VI	6th	18.0 sec.	98.6
	Recovered	11th	9.5 sec.	98.2
7	Pneumonia type III	7th	22.0 sec.	100.4
	Recovered	8th	25.0 sec.	100.2
		17th	16.5 sec.	99.0
8	Pneumonia type I	6th	13.3 sec.	103.0
	Recovered	10th	15.0 sec.	100.6
		14th	16.4 sec.	102.0
		17th	11.0 sec.	101.0
9	Pneumonia type I	2nd	20.0 sec.	106.2
	Recovered	5th	22.0 sec.	99.6
		11th	12.9 sec.	98.4
		15th	13.3 sec.	98.6
10	Meningitis	3rd	18.5 sec.	102.0
	Recovered	6th	12.5 sec.	98.6
11	Upper respiratory infection	2nd	19.0 sec.	100.8
	Recovered	6th	19.0 sec.	98.4
12	Pneumonia type III	2nd	14.6 sec.	103.0
	Died	3rd	16.1 sec.	103.0
13	Pneumonia type V	5th	15.2 sec.	104.0
	Died	6th	5.0 min.	102.8
14	Pneumonia type III	3rd	16.5 sec.	101.4
	Died	10th	12.2 sec.	101.2
		20th	12.2 sec.	102.0
15	Pneumonia type III	4th	20.0 sec.	104.4
	Died	5th	3 min. 14 sec.	104.0

TABLE II

RELATIVE FREQUENCY OF NORMAL AND ABNORMAL CIRCULATION TIME IN PATIENTS WHO RECOVERED AND THOSE WHO DIED

Only 11 patients who died had circulation time determinations done.

	PATIENTS RECOVERED		PATIENTS DIED	
	NUMBER	PER CENT	NUMBER	PER CENT
Normal Circulation Time	52	78.8	6	54.5
Prolonged Circulation Time	14	21.2	5	45.5

TABLE III

THE RELATIVE FREQUENCY OF ABNORMAL AND NORMAL CIRCULATION TIME IN INDIVIDUALS ABOVE AND BELOW 45 YEARS OF AGE

	BELOW 45 YEARS		ABOVE 45 YEARS	
	NUMBER	PER CENT	NUMBER	PER CENT
Normal Circulation Time	53	80.3	5	45.5
Abnormal Circulation Time	13	19.7	6	54.5

Normality or abnormality of the circulation time did not go hand in hand with corresponding changes in the electrocardiogram, as shown in Table IV. The circulation time was as frequently prolonged when the electrocardiogram was normal as when it was abnormal. Also, marked electrocardiographic abnormalities were sometimes seen when the circulation time was normal.

TABLE IV

COMPARATIVE FREQUENCY OF NORMAL AND ABNORMAL ELECTROCARDIOGRAMS ON DAYS WHEN THE CIRCULATION TIME WAS NORMAL OR ABNORMAL

	NORMAL ELECTROCARDIOGRAM		ABNORMAL ELECTROCARDIOGRAM	
	NUMBER	PER CENT	NUMBER	PER CENT
Normal Circulation Time	34	48.6	36	51.4
Abnormal Circulation Time	14	50.0	14	50.0

In pneumonia patients the type of organism appeared to have some effect on the frequency of electrocardiographic changes and the prolongation of the circulation time, as shown in Table V. The number of cases, however, was not sufficient from which to draw definite conclusions. No relationship was noted between electrocardiographic abnormalities and clinical signs of dyspnea, cyanosis, abnormal heart sounds and murmurs, as shown in Table VI. Abnormal electrocardiograms occurred just as frequently with as without these signs. Prolonged circulation time, on the other hand, occurred more frequently in the presence of these symptoms.

TABLE V

RELATIONSHIP OF TYPE OF PNEUMONIA TO NORMAL AND ABNORMAL CIRCULATION TIME AND ELECTROCARDIOGRAM

PNEUMONIA TYPE	CIRCULATION TIME				ELECTROCARDIOGRAM			
	NORMAL		ABNORMAL		NORMAL		ABNORMAL	
	NO.	%	NO.	%	NO.	%	NO.	%
I	6	85.7	1	14.3	3	37.5	5	62.5
II	6	75.0	2	25.0	5	62.5	3	37.5
III	4	57.0	3	43.0	3	50.0	3	50.0
V	3	100.0	0		2	66.6	1	33.3
VII	6	85.7	1	14.3	5	71.0	2	29.0
VIII	0		1	100.0	1	100.0	0	
XII	1	100.0	0		0		1	100.0
XX	1	100.0	0		1	100.0	0	
XXI	1	100.0	0		0		1	100.0
Untyped	27	87.0	4	13.0	12		22	

TABLE VI

RELATIONSHIP OF OBJECTIVE SIGNS TO FREQUENCY OF NORMAL AND ABNORMAL CIRCULATION TIME AND ELECTROCARDIOGRAM

PHYSICAL SIGNS	ELECTROCARDIOGRAM				CIRCULATION RATE			
	NORMAL		ABNORMAL		NORMAL		ABNORMAL	
	NO.	%	NO.	%	NO.	%	NO.	%
Dyspnea	19	48.7	20	51.3	29	80.5	7	19.5
No dyspnea	18	48.7	19	51.3	27	84.4	5	15.6
Cyanosis	16	53.3	14	46.7	22	81.4	5	18.6
No cyanosis	18	47.4	20	52.6	28	82.5	6	17.5
Murmurs or gallop	11	55.0	9	45.0	14	73.7	5	16.3
No murmurs or gallop	30	55.5	24	44.5	41	85.4	7	14.6
Abnormal heart sounds	21	48.8	22	51.2	37	84.0	7	16.0
Normal heart sounds	19	51.3	18	48.7	31	86.0	5	14.0

DISCUSSION

It appears from our observations that, although electrocardiographic changes frequently occur in infectious disease, they may not signify myocardial involvement. With the exception of rheumatic fever and rarely other active infections specifically involving the heart, changes in the electrocardiogram noted in the course of acute infectious disease might occur just as often in patients with or without demonstrable cardiac damage, as evidenced by clinical signs and circulation time determinations. It might be that changes are due in many cases to the febrile state itself and, in pneumonia, to such conditions as alterations in the environmental media of the heart and slight positional changes of that organ caused by solidified lung.

That fever in itself may produce changes in the electrocardiogram was demonstrated by Vesell and Bierman.[12] They explained the phenomenon by assuming that local cardiac and general chemical and physiologic alterations are produced by hyperthermia. Katz and his co-workers[13, 14] demonstrated changes in the electrocardiogram by alteration of the media adjacent to the heart. Sigler,[15] and previously Meek and Wilson,[16] Nathanson,[17] Kountz and Prinzmetal and others,[18] observed definite changes in the electrocardiogram by alteration in posture. We must, therefore, be on guard in evaluating electrocardiographic alterations in the course of acute infectious disease.

The circulation time appears to be a much better criterion of the presence of cardiac involvement in infectious disease. Our incidence of prolonged circulation time is somewhat greater than that of Hitzig, King, Bullowa, and Fishberg.[9] We included in the abnormal group, however, some patients who showed figures only slightly above the maximum limit of normal. The actual number of persons showing definitely prolonged circulation time would perhaps approach their figures.

It is of interest to find that prolonged circulation time occurred more frequently in patients who died than in those who survived. This would speak for cardiac failure as a contributing cause of death in a small proportion of cases. Although none of these patients presented signs of cardiac disease before, it is interesting to observe also that the circulation time is more often prolonged after 45 years of age, when degenerative changes are apt to occur.

Repeated determinations of the circulation time in the same patient revealed some variations in length from time to time. There was nearly always a tendency for it to become shorter on recovery from the disease, even if abnormally prolonged before. This would indicate that in an occasional case some degree of myocardial insufficiency exists during the course of the disease which returns to normal later.

SUMMARY AND CONCLUSIONS

Repeated electrocardiograms and circulation time determinations were made on 81 patients with acute infectious disease, 69 of whom were patients with lobar pneumonia. Frequent changes in the electrocardiograms were noted in the course of the disease or in convalescence, some trivial, others more pronounced. The degree of changes did not go hand in hand with the severity of disease, with the degree of clinical signs, or with the length of circulation time. The

circulation time was found to be prolonged more frequently in patients who died than those who survived, and after 45 years of age than before. It was also more frequently prolonged when abnormal clinical signs were present. In an occasional case there was prolonged circulation time during the height of the disease which returned to normal later.

The findings would tend to lead to the following conclusions: 1. Circulation time is a good method to determine the cardiac efficiency during the course of acute infectious disease. 2. The electrocardiogram is of questionable value in determining the myocardial state in such disease. 3. Cardiac failure is at least partly responsible for occasional deaths in acute infectious disease. 4. It contributes at times to symptoms. 5. Latent and previously undiagnosed cardiac disease often occurs in persons past 45.

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THE ABSENCE OF ACUTE EFFECTS OF SEX HORMONES*

MACDONALD DICK, M.D., DURHAM, N. C., AND CHARLES W. HOOKER, PH.D., NEW HAVEN, CONN.

ALTHOUGH much is known of the physiologic actions of the various estrogenic and androgenic hormones, there seem to be insufficient data to establish whether or not there are any immediate systemic effects which accompany a marked increase in circulating hormone. It has been reported[1] that intravenous or subcutaneous administration of crystalline menformon, although without effect in men, promptly resulted in a diminution of the blood pressure in hypertensive women, amounting to 15 to 20 mm. Hg; this substance rapidly lowered the blood pressure in female dogs but was inactive in this respect in male dogs. On the other hand, it has also been reported[2-4] that up to 10 R.U. of menformon or follicular extract have no effect on blood pressure or respiratory rate of dogs and cats, and that intravenous administration of quite large amounts of theelol produced no change in blood pressure, heart rate, and respiratory rate of anesthetized dogs.[5]

Steinach and his co-workers[6] have reported that testosterone and androsterone given subcutaneously reduced the blood pressure of patients after about two weeks. Greene[7] denies such an effect. In eunuchs, on the other hand, who are often hypotensive, testosterone seems to produce a rise in blood pressure and pulse rate.[8]

Since there apparently have been no studies of the immediate systemic effects of the androgens and since in the estrogen studies very small amounts have been given, we examined the effects of large doses of these hormones in 6 young adult dogs under morphine-sodium barbital anesthesia. Continuous records of two to six hours were taken of respiration by means of a pneumograph and of arterial blood pressure by a mercury manometer connected by a cannula to a carotid artery. The hormone preparations were injected directly into a cephalic vein. One female received 400 I.U. of theelin dissolved in 2 c.c. of water. One male and one female each received 50,000 I.U. of estradiol benzoate in 1 c.c. of sesame oil suspended in 2 c.c. of the animal's serum. One male and one female each received 50 mg. of testosterone propionate in 1 c.c. of sesame oil suspended in 2 c.c. of serum from the animal. The remaining animal, a male, received 45 mg. of powdered testosterone propionate suspended in 5 c.c. of the recipient's serum.

When an adequate record had been obtained, each animal was castrated, all wounds were closed, and the animal allowed to recover. After a rest period of three weeks the animal was subjected to the same procedures as

*From the Department of Physiology and Pharmacology, Duke University School of Medicine, Durham, and the Department of Anatomy, Yale University School of Medicine, New Haven.

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before in an attempt to ascertain whether there is any difference in the intact and castrated animal. On both occasions the cannula for recording blood pressure was put into the left common carotid artery.

In none of the animals was there any change whatever, either before or after castration, in heart rate, blood pressure, or respiration, either immediately on the intravenous administration of the hormone or up to six hours later. The blood pressures and respirations for each animal were essentially the same in the two experiments, although in the interval between measurements the animal had undergone gonadectomy as well as recovery from the first experiment.

In order to determine whether the vasoconstrictor mechanism was capable of normal response, each animal was given a small amount of epinephrine at the end of each recording. The rise of blood pressure was in every instance quite normal.

While it is possible that such factors as removal of the hormone from the oil, hydrolysis of the hormone esters, and excretion and inactivation of the hormones by the body may have altered the effective concentration, it is, nevertheless, clear that in these experiments we have increased the amount of androgen or estrogen in the circulation as abruptly as would be possible by any mode of administration. It follows that the administration of these substances is not accompanied, at least in the dog, by immediate systemic effects.

SUMMARY

Intravenous administration of 50 mg. of testosterone propionate, 50,000 I.U. of estradiol benzoate, or 400 I.U. of theelin to male and female dogs, both castrated and intact, under morphine-sodium barbital anesthesia produced no change in respiration, heart rate, or blood pressure.

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EVALUATION OF OXALATE SOLUTIONS FOR THE DETERMINATION OF PACKED CELL VOLUME IN HUMAN BLOOD*

JANE M. LEICHSENRING, PH.D., EVA G. DONELSON, PH.D., AND LUCILLE M. WALL, B.S., MINNEAPOLIS, MINN., AND MARGARET A. OHLSON, PH.D., AMES, IOWA

PACKED cell volume measurement, as a basis for the determination of the normality of an individual's blood picture, has been receiving increasing attention in recent years. The differences in the normal values reported in the literature may be attributed, in part, to the wide variation in the methods used. In the present study the Van Allen hematocrit was employed. As has been pointed out,[28] the accuracy of the measurement is dependent upon the amount of centrifugal force applied and on the kind and concentration of the anticoagulant used. This investigation is concerned with the latter as applied to human blood.

That the concentration of anticoagulant definitely affects the accuracy of measurement of the volume of the packed cells has been pointed out repeatedly by those who have used the method. Van Allen stated that the anticoagulant must be isotonic with the blood plasma; he also suggested the use of hirudin or heparin powder at the site of the removal of the sample, or even the use of no anticoagulant. Haden[13] similarly emphasized the importance of an isotonic anticoagulant.

Table I, which is a summary of anticoagulants that have been employed by other investigators, shows the wide variation in choices made, not only of kind but also of concentration.

METHODS

In the present study the procedure employed was essentially as follows: Blood samples were taken, through finger-tip puncture, from subjects who represented chiefly women of college age (17 to 25 years), with an additional group of older women whose ages ranged from 25 to 50 years. The latter were studied for the purpose of observing whether there was associated with age a demonstrable change in the tonicity of the blood which should alter the concentration of anticoagulant to be used with older individuals.

Another point investigated was whether having the subject in basal or non-basal condition should influence the concentration of oxalate solution to be used.

*From the Division of Home Economics, University of Minnesota, University Farm, Minneapolis, and the Division of Home Economics, Iowa State College, Ames.

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TABLE I

KIND AND CONCENTRATION OF ANTICOAGULANTS REPORTED IN THE LITERATURE

ANTICOAGULANT	PROPORTIONS TO BLOOD	INVESTIGATOR
None		Capps[4]
Defibrinated blood		Bie and Möller[1]
Hirudin		Froehlich[8]
		Gram and Norgaard[11]
Heparin		Dieckmann and Wegner[5]
		Walters[29]
Dry sodium oxalate	100 mg. to 10 c.c. blood	Larrabee[14]
Dry potassium oxalate	20 mg. to 10 c.c. blood	Osgood[19]
	10 mg. to 5 c.c. blood	Wintrobe[31]
	40 mg. to 10 c.c. blood	Wintrobe and Miller[32]
	50 mg. to 10 c.c. blood	Campbell[3]
Sodium oxalate (1.2%)	0.5 c.c. to 10 c.c. blood	Dulière and Adant[6]
Sodium oxalate (1.3%)	Van Allen hematocrit	Smith and Prest[25]
Sodium oxalate (1.4%)	2 c.c. to 10 c.c. blood	Haden[13]
Sodium oxalate (1.6%)	2 c.c. to 10 c.c. blood	Haden[12]
		Walters[30]
		Schlomovitz, Hedding, and Kehoe[22]
		Murphy and Fitzhugh[16]
Sodium citrate (3%)	0.5 c.c. to 4.5 c.c. blood	Gram[10]
Ammonium hydroxide (6 parts) and potassium oxalate (4 parts)	2 mg. to 1 c.c. blood	Bethell[2]

All subjects for whom the data are reported as being secured under basal conditions had been prepared for a basal metabolism test. For the data secured under nonbasal conditions, blood samples were obtained from the subjects without regard for consumption of food, physical activity, and time of day.

The anticoagulants selected for the present investigation included 1.6 per cent and 1.8 per cent potassium oxalate and 1.2 per cent sodium oxalate. Heparin was used as the control anticoagulant. Fresh solutions were made weekly and stored in the refrigerator. These solutions were allowed to come to room temperature before using. All oxalate solutions were tested and found to be neutral in reaction. In order to ascertain whether oven-drying of the oxalates before solutions were prepared would significantly alter the results, some samples were dried in a vacuum oven for two hours at 105° C. This procedure seemed desirable since it was planned to use the same lot of oxalate over a considerable period of time.

It was also thought advisable to validate the use of heparin as the control anticoagulant. This was accomplished by comparing the values obtained with heparin with those secured when no anticoagulant was employed. Celerity in sampling and centrifugation resulted in satisfactory determinations. A good grade of heparin was applied, dry, at the site of the puncture. The oxalate solutions under consideration were tested simultaneously, using the undried salts. All samples were prepared in duplicate and centrifuged for thirty minutes at 2750 r.p.m. in an International Centrifuge No. 2, with a head 13.5 cm. in radius. The rate of centrifugation with varying loads was checked by means of a revolution counter, and was found to yield at least 2750 r.p.m. with a maximum load.

RESULTS

Evaluation of Heparin as the Control Anticoagulant.—Although it is stated quite generally[15, 20, 21] that heparin does not influence cell size, we were unable to locate data to support this contention.

The observed mean differences in hematocrit values of the heparinized and oxalated samples from those to which no anticoagulant was added are presented in Table II. The observations represent determinations made on from 32 to 34 different individuals, all under basal conditions. These differences are as follows: for heparin, –0.22 per cent; for 1.6 per cent potassium oxalate, +2.70 per cent; for 1.8 per cent potassium oxalate, –2.43 per cent; and for 1.2 per cent sodium oxalate, +1.42 per cent.

TABLE II

DIFFERENCES IN HEMATOCRIT VALUES OBTAINED WITH HEPARIN AND OXALATE SOLUTIONS FROM VALUES WITH NO ANTICOAGULANT

	NUMBER OF		MEAN DIFFERENCE IN PER CENT	t	PROBABILITY
	SUBJECTS	OBSERVATIONS			
Heparin	33	38	-0.22	0.05	> 0.90
Potassium oxalate (1.6%) (undried)	32	32	+2.70	3.54	< 0.01
Potassium oxalate (1.8%) (undried)	34	38	-2.43	4.91	< 0.01
Sodium oxalate (1.2%) (undried)	32	32	+1.42	3.05	< 0.01

The significance of these differences was determined by applying the Student "t" test[27] to the paired data. For the heparinized samples this difference was found to be insignificant, whereas a significant difference was observed with each of the oxalate solutions. These results justified the selection of heparin as the control anticoagulant.

TABLE III

VARIATION OF DIFFERENCES BETWEEN DUPLICATE SAMPLES

NO ANTICOAGULANT			HEPARIN			POTASSIUM OXALATE (1.6%) UNDRIED			POTASSIUM OXALATE (1.8%) UNDRIED			SODIUM OXALATE (1.2%) UNDRIED		
NO. OF DUPLICATES	MEAN OF DIFFERENCES	STANDARD DEVIATION SQUARED OF DIFFERENCES	NO. OF DUPLICATES	MEAN OF DIFFERENCES	STANDARD DEVIATION SQUARED OF DIFFERENCES	NO. OF DUPLICATES	MEAN OF DIFFERENCES	STANDARD DEVIATION SQUARED OF DIFFERENCES	NO. OF DUPLICATES	MEAN OF DIFFERENCES	STANDARD DEVIATION SQUARED OF DIFFERENCES	NO. OF DUPLICATES	MEAN OF DIFFERENCES	STANDARD DEVIATION SQUARED OF DIFFERENCES
42	0.65	0.22	71	0.60	0.24	70	0.92	0.53	70	0.79	0.35	70	1.04	0.82

A second approach to the study of the efficacy of heparin as the control consisted in evaluating the variability of the duplicates within determinations for each of the anticoagulants used. This was done by computing the mean of the differences and the standard deviation squared of the differences between duplicates. The results are recorded in Table III. It may be observed

that the variation was least for the heparinized samples and those with no anticoagulant. Of the oxalated samples the variation of those with 1.8 per cent potassium oxalate most nearly approached that of the heparinized samples, followed by those with 1.6 per cent potassium oxalate, while those with 1.2 per cent sodium oxalate showed relatively greater variation due to wide differences in three pairs of samples. These data also support the use of heparin as the control anticoagulant, despite the fact that certain investigators[15, 21] report difficulty with its use due to the formation of small clots which interfere with the hematocrit reading.

As a measure of the magnitude of the difference between two determinations on the same individual, the workers at Iowa State College computed the analysis of variance[26] on the data obtained from a series of determinations on each of 11 subjects using three anticoagulants, heparin, 1.4 and 1.6 per cent potassium oxalate. The results of these computations are given in Table IV. It is evident that there is less difference between two determinations on the same individual than there is between the means for different individuals. A highly significant difference was observed between the effects of the anticoagulants.

TABLE IV

ANALYSIS OF VARIANCE OF SEVERAL ANTICOAGULANTS (IOWA STATE COLLEGE)

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE
Total	74	
Methods	2	29.74*
Individuals	10	6.78*
Interaction	20	1.89
Within individuals	42	2.18

*Highly significant.

A similar computation on a series of determinations made in this laboratory gave the values recorded in Table V. The same highly significant difference between methods was observed.

TABLE V

ANALYSIS OF VARIANCE OF SEVERAL ANTICOAGULANTS

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE
Total	115	
Methods	3	35.33*
Individuals	13	28.80*
Interaction	39	0.56
Within individuals	60	0.92

*Highly significant.

In the tables which follow the results obtained with oxalate solutions are expressed in terms of differences from heparin. Tables VI and VII record the data secured at the University of Minnesota and Table VIII those obtained at Iowa State College.

Comparison of Results With Dried and Undried Salts.—It may be observed from the mean differences in per cent between the values obtained with heparin and those with the various concentrations of oxalate employed, that oven-drying the salt resulted in all cases in solutions of greater concentration

TABLE VI

DIFFERENCES IN HEMATOCRIT VALUES OBTAINED WITH OXALATE SOLUTIONS FROM VALUES OBTAINED WITH HEPARIN

CONDITION OF SUBJECT	STATE OF OXALATE	POTASSIUM OXALATE (1.6%)					POTASSIUM OXALATE (1.8%)					SODIUM OXALATE (1.2%)				
		NUMBER OF		MEAN DIFFERENCE IN PER CENT	t	PROBABILITY	NUMBER OF		MEAN DIFFERENCE IN PER CENT	t	PROBABILITY	NUMBER OF		MEAN DIFFERENCE IN PER CENT	t	PROBABILITY
		SUBJECTS	OBSERVATIONS				SUBJECTS	OBSERVATIONS				SUBJECTS	OBSERVATIONS			
Basal	Undried	45	103	+4.53	16.78	< 0.01	40	55	−1.62	4.45	< 0.01	40	55	+2.34	6.89	< 0.01
Basal	Dried	24	24	+0.19	0.48	> 0.60						24	24	+1.19	2.55	< 0.02
Nonbasal	Undried	73	84	+5.47	14.02	< 0.01	75	83	−0.85	2.70	< 0.01	70	74	+2.43	6.52	< 0.01
Nonbasal	Dried	17	18	+2.37	3.27	< 0.01	10	10	−5.13	11.61	< 0.01	16	17	+2.17	4.25	< 0.01

TABLE VII

DIFFERENCES IN HEMATOCRIT VALUES OBTAINED WITH OXALATE SOLUTIONS FROM VALUES WITH HEPARIN FOR THE SAME INDIVIDUALS UNDER BASAL AND NONBASAL CONDITIONS

CONDITION OF SUBJECT	POTASSIUM OXALATE (1.6%)*				POTASSIUM OXALATE (1.8%)*				SODIUM OXALATE (1.2%)*			
	NO. OF SUBJECTS	MEAN DIFFERENCE IN PER CENT	t	PROBABILITY	NO. OF SUBJECTS	MEAN DIFFERENCE IN PER CENT	t	PROBABILITY	NO. OF SUBJECTS	MEAN DIFFERENCE IN PER CENT	t	PROBABILITY
Basal	35	+5.84	8.82	<0.01	33	−0.86	1.12	<0.27	33	+3.13	4.49	<0.01
Nonbasal	35	+5.56	5.93	<0.01	33	+0.02	0.66	>0.48	33	+3.43	4.42	<0.01

*Undried salts.

(Table VI). It is also apparent that all of the oxalate solutions tested, with the exception of the 1.6 per cent potassium oxalate prepared from the dried salt (subjects under basal condition), produced results which differed significantly from those obtained with heparin.

Since varying numbers of observations were made for the different solutions in this series, an additional study was undertaken to investigate more directly the effect of drying the salts upon the hematocrit values obtained. Using solutions of both the dried and undried potassium oxalate in the concentration of 1.6 per cent, duplicate samples were prepared from the same finger prick for each of 42 separate individuals. In this series the mean difference observed between the hematocrit values for the undried salt as compared with the dried was –3.78 per cent, an amount sufficiently great to be statistically significant ($P < 0.01$). It is obvious that the undried salts used in the present study contained sufficient moisture to alter significantly the concentration of solutions prepared from them.

On the other hand, data secured at Iowa State College show that, when the potassium oxalate solutions were prepared from a previously unopened bottle, oven-drying the salt did not influence the results. In a series on 12 separate individuals the mean difference between the hematocrit values for heparin and those for both the dried and undried 1.6 per cent potassium oxalate solutions was 1.4 per cent. It would appear, therefore, that salts from a previously unopened bottle may not require oven-drying, whereas salts from previously opened bottles obviously require oven-drying to insure consistent results.

Comparison of Results Under Basal and Nonbasal Conditions.—When the mean differences from heparin for the values of the various oxalate solutions with the subjects under basal conditions were compared with the mean differences with the subjects under nonbasal conditions (Table VI), there appeared to be a trend suggestive of an increase in the tonicity of the blood under nonbasal conditions. The apparent consistency of the results in this series suggested the desirability of undertaking a further study in which direct comparisons would be made on the same individual under basal and nonbasal conditions.

The anticoagulants used in this additional series were heparin, 1.6 per cent and 1.8 per cent potassium oxalate, and 1.2 per cent sodium oxalate, prepared from the undried salts. The observed mean differences from heparin in the hematocrit values under basal and nonbasal conditions, based on 33 to 35 subjects, are presented in Table VII.

It is evident from these data that the observed differences are of essentially the same magnitude, indicating that the tonicity of the blood was unaltered.

The differences in hematocrit values with two concentrations of potassium oxalate solution from those with heparin, as observed at Iowa State College, are given in Table VIII. The determinations were made on a group of 58 women students, ranging in age from 18 to 24 years, all of whom were under nonbasal conditions. The samples were centrifuged until maximum packing

was secured (forty or more minutes) in an instrument with a head 12.5 cm. in radius, which yielded 2464 to 2488 r.p.m. with the load used. The instrument was checked repeatedly with a revolution counter.

TABLE VIII

DIFFERENCES IN HEMATOCRIT VALUES OBTAINED WITH OXALATE SOLUTIONS FROM VALUES WITH HEPARIN (IOWA STATE COLLEGE)

NUMBER OF OBSERVATIONS	POTASSIUM OXALATE (1.4%)			POTASSIUM OXALATE (1.6%)		
	MEAN DIFFERENCE IN PER CENT	*t*	PROBABILITY	MEAN DIFFERENCE IN PER CENT	*t*	PROBABILITY
58	+6.53	10.64	< 0.01	+2.07	4.66	< 0.01

It is evident from the data presented that the values for the 1.4 and 1.6 per cent potassium oxalate solutions differed significantly from those secured with heparin. The observed mean difference in hematocrit values between those with heparin and those with the 1.4 per cent potassium oxalate was +6.53 per cent; and similarly, for the 1.6 per cent potassium oxalate, +2.07 per cent.

Comparison of Results From Two Age Groups.—In Table IX, a comparison is made of the results with two age groups, 17 to 25 years, and 26 to 50 years. It may be noted that with the 1.6 per cent potassium oxalate the mean difference from heparin for the younger age group was +5.49 per cent, and for the older age group, +5.47 per cent. Similarly, with the 1.8 per cent potassium oxalate,

TABLE IX

DIFFERENCES IN HEMATOCRIT VALUES OBTAINED WITH OXALATE SOLUTIONS FROM VALUES WITH HEPARIN FOR TWO AGE GROUPS*

AGE OF SUBJECTS	POTASSIUM OXALATE (1.6%)†				POTASSIUM OXALATE (1.8%)†				SODIUM OXALATE (1.2%)†			
	NO. OF SUBJECTS	MEAN DIFFERENCE IN PER CENT	*t*	PROBABILITY	NO. OF SUBJECTS	MEAN DIFFERENCE IN PER CENT	*t*	PROBABILITY	NO. OF SUBJECTS	MEAN DIFFERENCE IN PER CENT	*t*	PROBABILITY
17-25	33	+5.49	9.80	<0.01	30	−0.60	1.37	>0.17	30	+2.62	4.14	<0.01
26-50	35	+5.47	8.10	<0.01	36	−0.75	1.60	>0.10	36	+2.86	6.89	<0.01

*Subjects not in basal conditions.
†Salts not dried.

which was very slightly hypertonic, the mean difference for the younger age group was −0.60 per cent, and for the older age group, −0.75 per cent. From these data it is evident that there was no difference in the tonicity of the blood of these two age groups.

DISCUSSION

Although the importance of the concentration of the anticoagulant employed has been emphasized by a number of workers, comparatively few reports in the literature have evaluated anticoagulant solutions in terms of their tonicity with human blood. Furthermore, there appears to be considerable difference of opinion as to the concentration of solution of the various salts which are isotonic with human blood. Graff and Clarke[9] reported the iso-

tonicity of a solution of potassium oxalate between a concentration of 0.9 and 1.1 per cent. Haden, in his earlier work,[12] used 1.6 per cent sodium oxalate, but later[13] stated that 1.4 per cent sodium oxalate was isotonic with human blood and comparable, in its effect on cell size, to heparin and hirudin. Magath and Hurn[15] reported a much lower concentration of this salt (1.1 per cent) produced the same values as heparin. Norgaard and Gram[18] found that a 3.0 per cent sodium citrate solution did not alter cell volume, whereas Foster and Johnson[7] observed a 4.9 per cent shrinkage with a 1.3 per cent solution of the same salt. Such discrepancies are difficult to explain, except as being due to failure to control adequately all the conditions of the experiment.

In the present study commonly used concentrations of oxalate solutions were evaluated in terms of heparin. In addition, those factors which might explain the divergent results reported in the literature were investigated. The effect of drying the salt prior to preparation of the solution, the effect of the condition of the subject, that is, whether basal or nonbasal, and the effect of the age of the subject, were studied. Furthermore, the use of heparin as the control anticoagulant was affirmed from the standpoint of the absence of alteration in cell size and of the consistency in the results obtained.

The data secured in this laboratory show that oven-drying the salt before solutions are prepared is essential if the bottle has been previously opened, whereas the data from the Iowa State College laboratory demonstrate that if the solution is prepared from a previously unopened bottle of the salt, preliminary drying may be unnecessary.

It is obvious that any differences observed between the solutions which were prepared from the dried and undried salts cannot represent a constant relationship, since a number of factors may influence the amount of atmospheric moisture absorbed by the salt. The magnitude of these differences is such as to emphasize the importance of consistent oven-drying of the salts before preparation of solutions.

Of the solutions prepared from the dried salts, the 1.6 per cent potassium oxalate gave values which varied the least from the results secured with heparin. The 1.4 per cent potassium oxalate (Iowa State College data) and the 1.2 per cent sodium oxalate were sufficiently hypotonic to give results which differed significantly from the heparin values. The 1.8 per cent potassium oxalate, on the other hand, was hypertonic as compared with human blood, the difference again being significant.

There have appeared in the literature from time to time studies on the effect of exercise upon the composition of the blood. Changes reported, which might alter the osmotic pressure of the blood, include increases in lactic acid, serum protein, serum sugar, red blood cells, and hemoglobin.[17, 23, 24] In the present study the differences from heparin for the oxalate solutions were of like magnitude for the same subjects under basal and nonbasal conditions, indicating that changes which occur do not necessitate an alteration in the concentration of the anticoagulant employed.

SUMMARY

1. A study was undertaken for the purpose of evaluating several concentrations of oxalate solutions in terms of their tonicity with the plasma of human blood.

2. The subjects for this investigation consisted of a large group of college age women (17 to 25 years), with a smaller group of older women (26 to 50 years).

3. The use of heparin as the control anticoagulant was affirmed by a study in which the results from heparin were compared with those obtained when no anticoagulant was used. The accuracy of the results with heparin was demonstrated by the calculation of the variation between duplicate determinations.

4. Analysis of variance demonstrated a highly significant difference among the means of the packed cell volume values secured with the several oxalate solutions, and among the means of the individuals' packed cell volume values.

5. Preliminary oven-drying of the salt was shown to be essential in the preparation of the oxalate solution.

6. Of the various concentrations tested, 1.6 per cent potassium oxalate, prepared from the dried salt, was found to be most nearly isotonic with human blood. The 1.4 per cent potassium oxalate and the 1.2 per cent sodium oxalate were found to be significantly hypotonic, whereas the 1.8 per cent potassium oxalate was significantly hypertonic.

7. No differences were observed in the tonicity of the blood of subjects under basal conditions as compared with those same subjects under nonbasal conditions.

8. Within the limits of the ages studied, no differences in the tonicity of the blood were observed.

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FREQUENCY OF SYPHILIS IN OFFICE PRACTICE

HENRY G. HADLEY, M.D., WASHINGTON, D. C.

THE blood serum of 7000 consecutive office patients receiving physical examinations was tested by one or more methods for syphilis. Of the 7000 patients 4046, or 57.8 per cent, were female and 2954, or 42.2 per cent, were male. The average age was 41.91 years. Of this number positive serum reactions were obtained in 165, or 2.35 per cent. Of these 76, or 46.1 per cent, were females and 89, or 53.9 per cent, were males. The average age of this group was 34.33 years. The percentage of positives in the total group of the females was 1.8 per cent and of the males, 3.01 per cent.

Of the 7000 patients the Wassermann test alone was run on 4257 sera from 4244 patients, of which 76 were positive from 63 patients. The Kahn test alone was run on 946 sera from 870 additional patients, of which 76 were positive on 63 patients. The sera found positive by either test were run by the other method also. Two thousand one hundred and thirteen sera from 1886 patients were run with the Laughlen antigen in a comparative series with other tests, one to four other methods being run on the same serum sample. These results are as follows:

Laughlen 2113, No. positive 320
4+—177, 3+—62, 2+—55, 1+—26; Doubtful 1
Kline 1108, No. positive 269
4+—149, 3+—51, 2+—46, 1+—23; Doubtful 1
Kahn 376, No. positive 95
4+—66, 3+—16, 2+—9, 1+—4
Wassermann 1268, No. positive 154
4+—108, 3+—10, 2+—19, 1+—17
Hinton 133, No. positive 32
4+—17, 3+—0, 2+—6, 1+—9; Doubtful 1
Eagle 86, No. positive 24
4+—15, 3+—1, 2+—7, 1+—1
Rytz 60, No. positive 14
4+—11, 3+—2, 2+—1, 1+—0
Spinal fluid 17, No. positive 5

There is no relationship between the number of positives in the different methods employed, as the positive sera were repeated more often than the negative in the comparative tests.

There were 37 disagreements in this series, 25 of which were of no importance, being a minor difference in the reading of weakly positive bloods in treated cases. The 12 other disagreements are as follows:

1. Laughlen and Kline 2+, Eagle 4+
2. Laughlen 3+, Kline and Wassermann 2+, Hinton—Doubtful

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3. Laughlen 3+, Kline and Kahn Negative, Wassermann 2+
4. Laughlen and Eagle 2+, Kline and Wassermann 4+
5. Laughlen 4+, Kline 2+, Kahn—Negative
6. Laughlen 4+, Kline and Kahn 2+
7. Laughlen and Kline 3+, Kahn 2+, Wassermann—Negative
8. Laughlen and Kline 3+, Kahn 1+
9. Laughlen—Negative, Kahn—Doubtful, Wassermann (twice) 4+
10. Laughlen, Hinton, and Eagle 4+, Wassermann—Doubtful
11. Laughlen and Kahn 2+, Wassermann 4+
12. Laughlen and Kahn 1+, Wassermann 4+

Seventeen spinal fluids were run, of which 5 were positive. The Kahn and Kline each showed one more positive than the Laughlen, this being accounted for by the difference in method, the Kahn concentrating the spinal fluid and the Kline the antigen. Therefore, the Laughlen may not show a weakly positive spinal fluid.

In this series were 106 cases of pregnancy, 3 of which showed 4+ positive serum reactions.

Three hundred tests were performed on whole blood with the Laughlen antigen and two mistakes were found on rechecking with the use of serum, one false positive and one false negative. This method, therefore, was discontinued.

Two hundred tests were carried out on unheated sera with both the Laughlen and the Kline antigens, and one missed 4+ was found on rechecking. This method was considered entirely unreliable.

The method found to be satisfactory with the Laughlen antigen was the same as that used with the Kline antigen: heated serum on ringed glass slides.

It was found best to use two or more methods for each serum, and the use of either the Kline and the Laughlen or the Hinton and the Laughlen tests was very satisfactory. The Kline and Laughlen methods were used more often because of the convenience of having two tests using almost identical methods and because the result may be read immediately.

CONCLUSION

In an unselected series of white patients presenting themselves for physical examinations in an office practice devoted to internal medicine, 7000 routine tests showed 165 positive for syphilis, or 2.35 per cent. The Laughlen antigen used in 2108 tests on 1883 patients gave equally as good results as any of the other methods employed.

DENTAL COÖPERATION IN DIABETES MELLITUS*

SAMUEL BLAUSTEIN, D.D.S., F.A.D.S.M., AND EDGAR FERGUSON
BROOKLYN, N. Y.

A GROUP of 125 patients with diabetes mellitus were treated in the dental department and 252 teeth were extracted. This included single and multiple extractions as well as cyst removals. A study was made from both a diabetic standpoint as well as a dental one. The diabetic study included diet, insulin dosage, and blood sugar studies (182 in number) before and after the extractions. The dental study included dental treatment, observation of the rate of healing of these patients as compared with nondiabetic persons, and observations of postoperative sequelae when present.

When observed from a diabetic standpoint, these patients may be classified on the long-time basis of insulin dosage. It was found that there were 62 mild (from 0 to 20 units of insulin) cases treated, 16 medium (from 20 to 40 units), and 13 severe (from 40 to 65 units). Other than usual postoperative bleeding occurred in one patient in the severe range (7.7 per cent), in one patient in the medium range (6.2 per cent), and in 3 patients in the mild range (5 per cent). Infected granulation tissue occurred in one of the mild cases (1.6 per cent).

Another classification from the diabetic standpoint may be made from the blood sugar level at the moment of extraction. This was taken in 91 cases. There were 36 cases in the 100 to 150 mg. range, 35 cases in the 150 to 250 mg. range, and 20 cases between 250 and 350 mg. range. Four cases of bleeding occurred in the low blood sugar range (11 per cent), none occurred in the medium range, and only one occurred in the high range (5 per cent). The case of infected granulation tissue fell into the medium range.

It was found that dental surgical intervention did not cause any significant change in the blood sugar when taken immediately following the operation. The average rise or fall did not exceed 25 per cent of the original blood milligram figure, and this is within the individual error of the micro-blood-sugar method. No extreme blood sugar rise was observed, and no patient experienced insulin shock while under treatment.

Significant from the diabetic viewpoint was the fact that the time of operation was between 10:00 and 11:30 A.M., which is one and one-half hours following insulin and breakfast. At this point, the level of the blood sugar is higher than at other times of the day but descending. This at once precludes the possibility of insulin shock and untoward rise.

*From the Metabolic Clinic, Kings County Hospital, Brooklyn. Henry M. Feinblatt, M.D., Director.

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The conclusions on the diabetic observations are: 1. The percentage of bleeding bore no relation to the severity of the diabetes when calculated on the basis of insulin dosage, or to the blood sugar level at the time of operation. 2. The dental interference did not cause any significant change in the level of blood sugar when the operation was concluded within two hours after breakfast and insulin.

When observed from a dental standpoint, the first consideration is the rate of healing as compared to the normal. There were only 4 cases of bleeding in a total of 125 cases observed (3.2 per cent), which is not greater than normal. Swelling occurred in 3 cases and infected granulation in one case.

Further dental considerations include the choice of anesthetic. No untoward effects due to anesthesia were observed, except nausea in 2 cases and vomiting in one case. These untoward effects occurred with nitrous oxide-oxygen and bore no relation to insulin dose or blood sugar level (2 in the medium and 1 in the high range). No such effects occurred in any range with the three types of local anesthesia used (2 per cent procaine with no epinephrine, 2 per cent procaine with 1:25 M epinephrine, and monocaine with 1:75 M epinephrine). In a person with diabetes, vomiting and nausea is of significance since his successful diabetic treatment depends on a close balance between carbohydrate absorption and insulin action. If a disturbance of this balance can be avoided, the regular diabetic routine will not be disturbed.

The conclusions from the dental point of view are: 1. The percentage of postoperative sequelae was not higher than normal. 2. General anesthesia is contraindicated due to the fact that it is prone to cause vomiting which interferes seriously with the diabetic nutrition. 3. Of the three types of local anesthesia used no significant difference in effect was observed in the diabetic patient.

CHRONIC COR PULMONALE OF UNUSUAL ETIOLOGY*

LAUREN V. ACKERMAN, M.D., RUTLAND, MASS.

THE case of chronic cor pulmonale here reported shows primary changes in the pulmonary artery and its smaller radicles with secondary pulmonary fibrosis and right heart failure. The changes in these arteries as well as others showed both acute and chronic inflammation.

CASE REPORT

Clinical History.—The patient was a 45-year-old housewife who was admitted to the Rutland State Sanatorium on Dec. 3, 1937. Her past history was irrelevant, except for an unknown illness at the age of 11. She had been diagnosed at that time as having pulmonary tuberculosis. She had had no symptoms and nothing which would suggest such a diagnosis after that illness. Her present illness began at some indefinite time during the past eight or ten years. She had a chronic cough of several years' duration productive of small quantities of sputum which was never foul. She began to have intermittent attacks of dyspnea associated with some cyanosis. During an attack which was characterized by slight intermittent fever and quite marked dyspnea, the cyanosis would become more pronounced.

At the time of her entry here a physical examination showed a fairly well developed, well nourished, white adult female. She was extremely cyanotic. There was no clubbing of the nails, skin nodules, or rash noted. The principal signs were in her lungs, with dullness at the right base and occasional râles. The heart was not enlarged, and the sounds were distinct. P_2 was greater than A_2. There were no murmurs. The blood pressure was 130/70. She also had a quite marked scoliosis of the dorsal spine. The spleen and liver were not palpable. The clinical laboratory findings were all negative. Her sputa were negative. There were no tubercle bacilli or fungi found. A Hinton test was negative. The urine showed a slight trace of albumin, and the blood was normal except for leucocytosis with a differential count characteristic of an acute infection. There was no eosinophilia or polycythemia. Agglutination tests for undulant fever, paratyphoid A and B, and typhoid were negative. Three blood cultures were also negative. An electrocardiogram taken two months before admission showed slight right axis deviation. An x-ray taken Jan. 6, 1938, showed some mottling at the right apex, with obliteration of the right costophrenic angle. The left was clear, except for accentuated markings.

The clinical course was characterized by progressive dyspnea and cyanosis. She continued to run only a low-grade fever and at times had intermittent pains in her chest. Her cyanosis began to deepen and oxygen therapy was instituted. This seemed to give no relief. She also began to have auricular fibrillation which cleared soon after digitalization. Another x-ray of her chest on Feb. 5, 1938, showed an infiltrative process radiating out from the left hilum which was not present in the previous film. Mottling at the right apex and at the right base which had been present was about the same. In spite of supportive measures she died of acute respiratory insufficiency with signs of right heart failure.

Autopsy.—The anatomic diagnoses were acute and chronic arteritis of lungs, kidneys, ileum, gall bladder, spleen, periadrenal adipose tissue, vasa vasorum of pulmonary artery, pulmonary fibrosis, congestion of left upper lobe, bronchiectasis of right lower lobe, chronic fibrous and acute fibrinous pleuritis, bronchopneumonia, perisplenitis, and leiomyomas of uterus.

*From the Pathological Laboratory of the Rutland State Sanatorium, Massachusetts Department of Public Health.

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The body was fairly well developed. There was evidence of weight loss and scoliosis of dorsal spine was present. The heart weighed 500 gm., and the right ventricular wall measured 0.6 cm. and showed definite hypertrophy. The pulmonary artery was wrinkled and looked not unlike the tree bark wrinkling seen in syphilis. The aorta and other arteries examined appeared grossly normal. The abdominal organs showed cloudy swelling. The lungs were of the greatest interest. The right side of the lung cut with a great deal of difficulty, and streaks of gray fibrous tissue were present. In the lower lobe of the right lung there was a small patch of bronchiectasis. The pleural cavity on the right was obliterated. On the left there was an apparent recent pneumonitis in the lower lobe of the left lung, while the upper lobe showed fibrosis and some congestion. The cause of this marked pulmonary fibrosis was not apparent at the gross examination. The pleura of the left side of the lung showed an acute reaction.

Microscopic Examination.—Microscopically the heart muscles showed a few collections of cells around blood vessels; however, these were definitely not Aschoff's bodies. The pulmonary artery showed interruption of the elastica and periarterial collection of cells about the small arteries of the vasa vasorum. These arteries showed marked narrowing. The lung showed extensive fibrosis throughout. Marked changes in the small arteries were present. There were recent areas of necrosis in the media of a few small vessels, and around these vessels there were collections of polymorphonuclear cells with a few round cells. There were no eosinophiles seen. There was evidence of an older process and obliteration of some of these small arteries with recanalization of others. Some of them were completely obliterated. In the wall of the gall bladder there was also necrosis of the media of a small artery in the typical horse-collar arrangement that one sees in periarteritis nodosa. Around this small artery there was a collection of cells typical of an acute exudate. There were similar changes in the arteries about the adrenal and ileum. In the kidney the picture was not unlike that seen in focal embolic glomerular nephritis. Small thrombi were present in glomerular tufts. Wire loop lesions, such as Baehr[1] described in disseminated lupus, were not present. The pathology was patchy, no large vessels were involved, and no infarctions were present.

DISCUSSION

Clinically the diagnosis suggested chronic cor pulmonale with an obscure etiology. Tuberculosis was thought of at first because of the mottling at the right apex. Malignancy primary in the lung was also considered. Undulant fever, typhoid, subacute bacterial endocarditis, and other more obscure disorders were considered and discarded. A consultant in chest diseases called it tuberculosis. At the time of death there was no definite diagnosis.

Chronic cor pulmonale is a condition which has been described in detail in the literature. It is of manifold etiology with common causes, such as mitral stenosis, extensive pulmonary fibrosis, and emphysema secondary to asthma, chronic bronchitis, pneumoconiosis, or some other pulmonary disease. Rare causes, such as congenital cardiac defects, organic tricuspid stenosis, sickle-cell anemia, primary arteriosclerosis, or syphilis of the pulmonary artery and its branches have been described.[2-7]

In this particular case the pulmonary fibrosis was secondary to an acute and chronic inflammatory process beginning in the arterial system. Acute arteritis was present with acute inflammatory changes about the vessels and in the alveoli. As these acute changes subsided on previous occasions, chronic changes in the vessels as well as the pulmonary parenchyma occurred, narrowing of the lumina of small arteries and arterioles took place with, at times,

Fig. 1.

Fig. 2.

Fig. 1.—Necrotizing arteritis in small artery in wall of gall bladder. Photomicrograph ×200.
Fig. 2.—Chronic arterial changes in lung with acute pleuritis. Photomicrograph ×48.

Fig. 3.

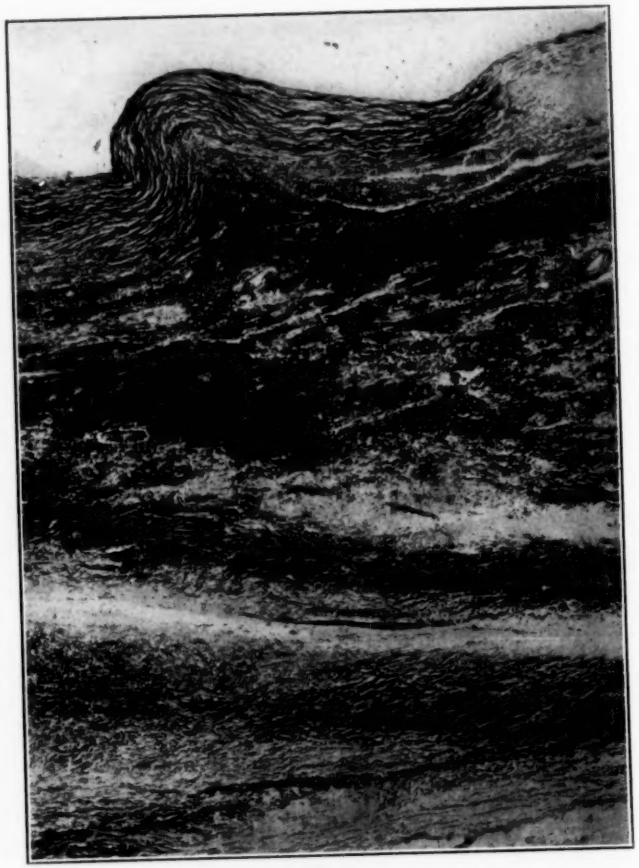

Fig. 4.

Fig. 3.—Extensive fibrosis in lung. Chronic arteritis. Bronchiectasis. Photomicrograph ×48.
Fig. 4.—Pulmonary artery. Note wrinkling of intimal surface and disruption of elastica. Photomicrograph ×48.

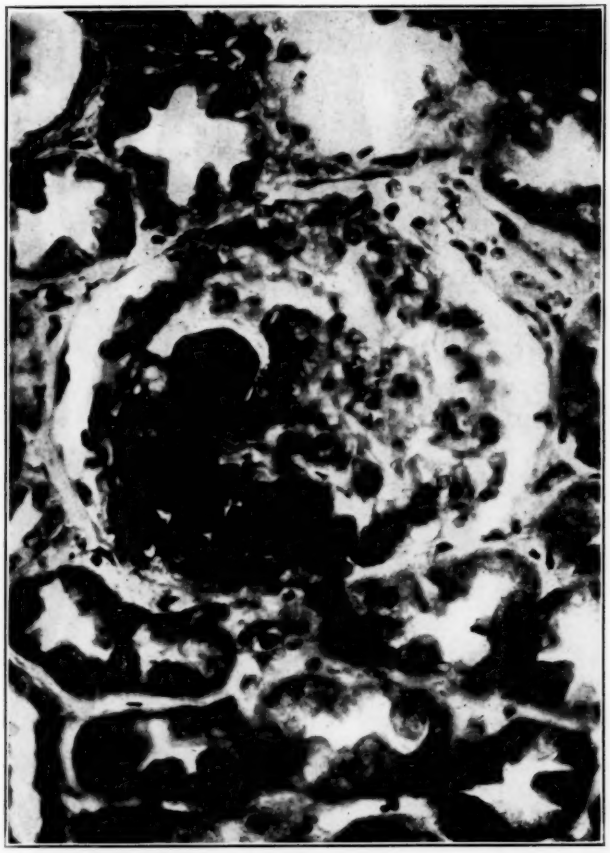

Fig. 5.—Glomerulus showing thrombus. Photomicrograph ×200.

obliteration and recanalization. These changes spread to the pleura and the pericardium. It is obvious that repeated attacks took place, causing more and more replacement of the lung with fibrosis. Her last attack proved too much for her cardio-respiratory system, and she died of failure of the right side of the heart. Changes in small arteries in other organs caused no impairment of function and apparently no symptoms.

Certain conditions could be definitely ruled out as a cause for this arteritis. It was not rheumatic in origin, for there was no definite history, obliteration of the lumen of the vessels does not take place in rheumatic involvement of arteries, and there were no Aschoff's bodies in the myocardium and no valvular lesions were present. It was certainly not syphilitic in origin, for there was no history of syphilis; the Hinton test was negative, the aorta was normal, and the other organs showed no evidence of syphilis.

This patient was also discussed from the viewpoint of disseminated lupus erythematosus. The clinical picture in a female with its chronicity, associated with inflammation of synovial membranes, pleura and pericardium, together with the widespread vessel involvement, suggested it. However, as Baehr and Klemperer[8] pointed out, the changes in the kidneys were not typical;

there was no thrombopenia, skin rash, nor endocarditis. It is of interest that in Mallory's[9] experience many fatal cases do not show the wire loop lesion of the kidney.

Periarteritis nodosa was strongly considered. Vessels of multiple organs were involved. There was no eosinophilia either in the circulating blood or about the vascular lesions. Infarctions and skin nodules were not present, but none of these conditions are essential. It is rather rare in the lungs. Herman[10] reported a single patient with rapidly progressive dyspnea, who on autopsy showed necrotizing arteritis and multiple areas of infarctions in the lung. Ophuls,[11] in his review, mentioned one patient who showed lung involvement. King[12] cited a patient in whom the x-ray and gross pathologic picture appeared like miliary tuberculosis, but who on microscopic examination showed miliary necrotizing arteritis. Gross and Friedberg,[13] in their discussion of periarteritis nodosa associated with rheumatic heart disease, discussed two patients who had involvement of arteries of the lungs.

Karsner[14] has well reviewed the situation existing today in relation to the etiology of necrotizing arteritis. The bacterial and parasitic agents known to cause necrotizing arteritis are many. Other agents, such as trypan blue,[15] allylamine,[16] injections of specific serum,[17] and allergic conditions[18] also cause this pathologic alteration in vessels. Periarteritis nodosa, a clinical entity which is not well defined because of its unknown etiologic agent, has certain criteria which some pathologists[14] believe noteworthy. These include the eosinophilia in the circulating blood or about the local vascular lesions and the presence of nodules formed because of the weakened vascular wall. The involvement of a large artery, the pulmonary in our case, the absence of eosinophilia either about the lesion or in the circulatory blood, the absence of nodules and infarctions, seem to rule out such a diagnosis if we accept the original description of the disease and adhere to criteria set up by Rokitansky,[19] Karsner,[14] and other pathologists for such a diagnosis.

We are left then with a necrotizing arteritis of unknown etiology. The small patch of bronchiectasis in this case was probably secondary to the fibrosis. There is no basis for syphilis, rheumatic fever, allergic conditions, or parasitic infection. The case apparently does not fit the poorly defined syndromes of either periarteritis nodosa or disseminated lupus erythematosus. It may be due to unidentified bacteria or their products, but we have no proof of it. While the clinical and pathologic findings are of extreme interest, we must not be misled by these structural alterations. It would be much more important to know the mechanism which set these processes in motion.

CONCLUSIONS

1. A case of widespread, acute, and chronic arteritis of unknown etiology is reported.

2. The most marked changes occurred in the pulmonary arterial circulation with secondary pulmonary fibrosis. These changes resulted in chronic cor pulmonale.

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AN EXPERIMENTAL STUDY OF THE RECTAL ADMINISTRATION OF MERCURIAL DIURETICS*

I. J. Brightman, M.D., and Robert A. Lehman, Ph.D., New York, N. Y.

WITHIN the last few years the administration of mercurial diuretics in the form of cocoa butter suppositories has become rather widespread, and many clinical reports have appeared concerning their effectiveness and local toxicity. From a recent review of the literature[1] it seems evident that both of the diuretics which are available in this country as suppositories (mercurin and salyrgan) are reasonably effective. However, it has been observed that both will occasionally give rise to burning and rectal tenesmus, and proctoscopic evidence of congestion of the rectal mucosa has been reported by Crawford[2] and by DeGraff, Cowett, and Batterman.[3] Furthermore, these authors noted several instances of ulceration after the use of salyrgan but none after the use of mercurin. Although the factors which control rectal irritation are not fully known, it seemed possible that by varying slightly the composition of the salyrgan or mercurin suppository, a preparation might be obtained which would be well tolerated and still retain the character of effective diuretic with low general toxicity peculiar to organic mercury compounds of this type. It was, therefore, decided to attempt to develop an experimental procedure for the comparison of suppositories of different chemical composition, both as to absorption from the rectum and as to local toxicity, and to apply it to the study of modified forms of salyrgan and mercurin.

Suppository masses of various compositions were furnished by the manufacturers† in a concentration in cocoa butter convenient for experimental use. The salyrgan suppository has been modified in three ways, by addition (1) of theophylline; (2) of the acid of which salyrgan is the sodium salt; and (3) of both. The introduction of theophylline was suggested by pharmacologic work which demonstrated that the local toxicity is decreased[4] and the rate of absorption increased[5] by its presence. On the other hand, since mercurin consists of a mixture of acid and sodium salt and appears to be superior with regard to local toxicity, it seemed logical to determine the effect of adding salyrganic acid to salyrgan. The composition of the various suppositories used is given in Table I.

*From the Department of Therapeutics, New York University College of Medicine.
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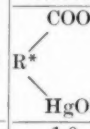
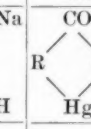
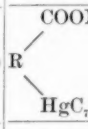
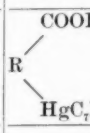
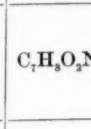
†Salyrgan, NNR, is manufactured and distributed by Winthrop Chemical Co., Inc.; mercurin, NNR, is manufactured by Chinoin Chemical and Pharmaceutical Works, Ltd., and distributed by Campbell Products, Inc.

TABLE I

COMPOSITION OF SUPPOSITORIES

CODE NO. OF EXPERIMENTAL SUPPOSITORIES	MG. OF MERCURY PER 500 MG. DOSE (BY ANALYSIS)	RELATIVE MOLE EQUIVALENTS				
		SALYRGAN OR MERCURIN SODIUM	SALYRGANIC OR MERCURINIC ACID	SALYRGAN SODIUM THEOPHYLLINE	SALYRGANIC ACID THEOPHYLLINE	FREE THEOPHYLLINE
						
Salyrgan	17.6	1.0				
618-3	20.0			1.0		
619	18.2			1.0		0.3
ST384†	20.0			1.0		0.3
S381, 618-2	16.2	0.5	0.5			
ST382	20.0	0.3		0.2	0.5	
ST3815	20.0			0.5	0.5	
ST3825	20.0				1.0	
Mercurin	17.9	0.8	0.2			
Mercurinic acid	20.0		1.0			

*For the structure and chemistry of salyrgan and mercurin, see Collins,[6] Moeller,[7] DeGraff,[8] and Lehman.[8]

†With sodium carbonate to a pH of 8.6.

PROCEDURE FOR THE COMPARISON OF TOXICITY OF SUPPOSITORIES

Cats weighing between 2 and 4 kg. were used as experimental animals. In order to standardize conditions and eliminate as much as possible the variable factor of the amount and consistency of the feces, the bowel was evacuated by oral administration of 35 c.c. of a 15 per cent solution of magnesium sulfate on two successive days. This assures soft and fairly uniform fecal material which would not be expected to interfere with the action of the suppository. The experiments were never started until forty-eight hours after the last dose of cathartic to allow any possible irritability of the bowel to subside. During this interval the animals were starved. The results of control experiments are given in the section on pathology and in Table III.

On the day of the experiment the cats were strapped to animal boards and anesthetized with 1.5 to 2.0 c.c. (0.10 to 0.13 gm.) of pentobarbital sodium intraperitoneally according to size. The suppositories were made up as small cylinders (about 6 mm. in diameter) so that 250 mg. of suppository contained 10 mg. of mercury regardless of the preparation. The doses usually given were equivalent to 10 or 20 mg. of mercury, but the concentration was always the same. This corresponds approximately to the dosage used clinically. As soon as the animals were deeply anesthetized, the suppositories were inserted by means of a glass cannula (outside diameter 1 cm.) and closely fitting plunger, pointed at one end and blunt at the other. The cannula and plunger with pointed end forward were introduced together; the shape of the end tended to overcome any spasm of the anal sphincters. The plunger was withdrawn, the suppository inserted into the cannula and pushed into place with the blunt end of the plunger.

The cannula and plunger were then carefully removed. By this procedure it was possible to place the suppositories consistently at about 6 cm. above the anal margin. Retention was 100 per cent.

The animals were killed by chloroform inhalation twenty-four hours after the insertion of the suppositories. The pelvic bone was then resected and the large intestine removed. The specimens were opened longitudinally and the feces removed under running water. Those which showed gross pathologic changes were fastened to glass frames, immersed in isotonic salt solution, and photographed on panchromatic plates. They were then fixed in Pick's solution and preserved as museum specimens. After fixing, histologic sections were made of any areas which seemed of interest.

PATHOLOGY

It was noted from the beginning that any of the preparations studied was capable of producing in the lower portions of the colon of the cat any of the pathologic changes to be described. However, as each series of experiments was enlarged, definite differences were found in the frequency with which lesions were produced and in the extent of involvement of the gut.

Controls.—The colons of 71 cats were examined as control studies for the experimental work. Of these, 55 had received no treatment related to the gastrointestinal tract, but had been sacrificed by digitalis assays. Fifty-four were entirely normal, while one exhibited a superficial yellowish exudate covering the distal 6 cm. of the bowel. This exudate was unavoidably detached during the preparation of the histologic section, and the microscopic examination revealed a normal gut wall. Five additional control cats received magnesium sulfate orally alone and 11 received the cathartic and also a 500 mg. nonmedicated cocoa butter suppository. Of these 16 one showed a thin exudate similar to that just described. Only 2 of 71 control cats revealed any unusual features in the large intestine and these were of minor degree. It was thus demonstrated that the procedure involved in preliminary magnesium sulfate catharsis and the introduction of the suppository by means of the glass cannula did not produce lesions in the bowel wall. Slight degrees of congestion were seen in both control and experimental animals but these were not regarded as significant.

Gross Examination.—The lesions observed in the experimental animals treated with mercurial suppositories were found exclusively in the lower 12 cm. of the large bowel and could broadly be divided into two main types, exudative and necrotic. The exudative lesions varied in type from a yellow-white granular, fibrinous, superficial covering to a thick yellow or green mucoid coat which was more or less firmly attached to the gut wall (as in Fig. 1 *A*). In a few cases a distinct pseudomembrane was formed. The exudate covered between 2 and 12 cm. of the distal length of the gut. The necrotic lesions appeared as brownish or purplish, well-demarcated, irregular, slightly depressed areas, varying from 2 to 12 mm. in diameter (Fig. 2 *A*). In addition to the exudative and necrotic lesions, occasional superficial hemorrhages were noted, varying from scattered punctate lesions to larger, poorly defined areas up to 10 cm. in diameter.

Fig. 1 *A*.—Gross specimen of lower half of colon of cat which had received suppository containing mercurial diuretic (Cat No. 155; dose of salyrgan equivalent to 10 mg. mercury). The exudate is extensive, yellow white, and mucoid in consistency, and is firmly attached to the gut wall. Small necrotic areas are also present but are hidden by the exudate. The pathology exhibited by this specimen was recorded as, exudate, 3 plus; necrosis, 3 plus.

Fig. 1 *B*.—Microscopic section through the specimen shown in Fig. 1 *A*. The mucosa is necrotic and partially sloughed off to form part of the overlying exudate which is composed of polymorphonuclear leucocytes and large mononuclear cells. There is considerable cellular infiltration of the submucosa.

Fig. 2 *A*.—Gross specimen of the lower half of colon of cat which had received a suppository containing a mercurial diuretic (Cat No. 108; dose of S381 equivalent to 10 mg. mercury). The necrotic areas are 2 to 10 mm. in size, purple red, well demarcated and slightly depressed. The pathology exhibited by this specimen was recorded as, necrosis, 3 plus.

Fig. 2 *B*.—Microscopic section through specimen shown in Fig. 2 *A*. The glandular architecture can be defined, but the cells have undergone coagulation necrosis and epithelial nuclei are not visible. There is considerable cellular infiltration of both mucosa and submucosa consisting chiefly of polymorphonuclear leucocytes.

For comparison of the toxicity of the various drugs employed, the extent of the lesions was graded as one to three plus. The exudative lesions usually encircled the gut and it was therefore possible to compare them by linear measurement. Involvement of less than 2 cm. of the length of the large intestine was considered as one plus; from 2 to 6 cm., two plus; and more than 6 cm., three plus. The necrotic and hemorrhagic lesions were grouped together. A total area of involvement of less than 0.25 sq. cm. was considered one plus; from 0.25 to 1.0 sq. cm., two plus; and more than 1.0 sq. cm., three plus. On the whole, it was possible to adhere to these criteria closely, but occasionally exact measurement was difficult and required subjective judgment.

Microscopic Examination.—Histologic preparations were made by the paraffin block method and stained with hematoxylin and eosin. In a few cases where gross sections had shown lesser degrees of exudation, the inflammatory material was apparently dissociated from the gut wall during the making of the preparation and no alterations were visible under the microscope. In others, however, definite inflammatory exudate could be seen overlying the mucosa, consisting in varying proportions of polymorphonuclear leucocytes and large mononuclear cells embedded in amorphous pink-staining debris. In the more severe degrees of involvement the mucosa appeared necrotic and from one-third to the entire thickness was sloughed off to form part of the overlying exudate (Fig. 1 *B*). In the submucosa there was congestion of the arteries and veins and an increased cellularity consisting chiefly of mononuclear cells. In one section a small area of hemorrhagic extravasation appeared at the base of the necrotic mucosa.

Section through areas of necrosis revealed considerable alteration of the mucosa and submucosa (Fig. 2 *B*). The architecture of the mucosal glands could be defined to some degree, but the cells had undergone coagulation necrosis and nuclei were no longer visible. The glandular structure appeared to be an amorphous pinkish mass. An infiltration of polymorphonuclear cells was present throughout the mucosa and underlying submucosa and the submucosal arteries were packed with cells.

PROCEDURE FOR THE COMPARISON OF SUPPOSITORIES WITH RESPECT TO ABSORPTION AND MERCURY EXCRETION

For estimating absorption and excretion the animals were prepared exactly as for the study of local toxicity. However, just before administration of the suppository a catheter was inserted, fastened in place with adhesive tape, and all subsequent urine collected in a flask. The specimens were discarded if a bowel movement occurred during the experimental period. After twenty-four hours the entire large intestine was clamped at either end and excised. The twenty-four-hour urine specimens and the unopened gut were then analyzed by the method of Winkler,[9] modified by Gettler and Lehman.[10] The procedure for the digestion of the intestine and contents was the same as that described by DeGraff, Batterman, and Lehman.[5] Test analyses on feces and intestine by this method have not been reported and are, therefore, given in

TABLE II

TEST ANALYSES ON LARGE INTESTINE AND CONTENTS

MG. MERCURY TAKEN	MG. MERCURY FOUND	RELATIVE PER CENT ERROR
4.99	4.57	- 8
3.49	3.27	- 6
2.49	2.47	- 1
1.47	1.31	-11
0.50	0.54	8

TABLE III

PROTOCOLS FOR EXPERIMENTS ON THE TOXICITY OF MERCURIAL DIURETIC SUPPOSITORIES

CODE NO. OF PREPARATION	TOTAL NO. OF ANIMALS	NO. OF ANIMALS SHOWING ONE PLUS REACTION OR MORE IN EITHER CATEGORY	NO. OF ANIMALS SHOWING EACH DEGREE OF MUCOUS OR FIBRINOUS EXUDATE			RATIO TOTAL PLUS REACTIONS TO NO. OF ANIMALS	NO. OF ANIMALS SHOWING EACH DEGREE OF NECROSIS OF INTESTINAL WALL			RATIO TOTAL PLUS REACTIONS TO NO. OF ANIMALS
			1 PLUS	2 PLUS	3 PLUS		1 PLUS	2 PLUS	3 PLUS	
Controls:										
1. No treatment	54	1	-	1	-		-	-	-	
2. Catharsis alone	11	-	-	-	-		-	-	-	
3. Catharsis and cocoa butter suppository	9	1	-	-	1		-	-	-	
ST3815	18	3	-	3	-	0.33	-	1	-	0.11
ST382	18	5	-	4	1	0.61	1	-	-	0.06
619	12	4	1	2	1	0.67	1	1	-	0.25
618-3	23	12	4	2	5	0.91	2	2	-	0.26
Mercurin	30	14	3	4	5	0.87	3	3	1	0.40
ST384	12	8	-	4	2	1.17	2	3	-	0.67
Salyrgan	37	23	3	8	9	1.25	2	5	5	0.73
S381, 618-2	20	15	1	7	3	1.20	3	3	6	1.35

Table II. These were carried out by analyzing specimens from normal cats to which known amounts of mercuric nitrate had been added. It will be seen that the accuracy is adequate for the purposes of this study.

RESULTS AND CONCLUSIONS

In Table III the protocols are given for the experiments on the local toxicity of suppositories. A separate classification has been made for the two types of lesions observed since they are distinct and may be found either alone or together. For comparative purposes arbitrary numbers have been derived to indicate the relative extent and frequency of each type of reaction. The number of animals showing one-, two-, or three-plus exudative reactions was multiplied in each case by one, two, or three, and the combined total of "plus" scores in turn divided by the number of animals used. Thus, for mercurin the number 0.87 was obtained: $[(3 \times 1) + (4 \times 2) + (5 \times 3)] \div 30$. This process was repeated for the necrotic lesions. The ratios thus obtained were divided by the smallest of the series in either category (i.e., the ratio for compound ST3815). These numbers appear in columns 2 and 3 of Table IV. In column 4 of Table IV are listed the percentages of animals showing a one-plus reaction or greater of any type.

TABLE IV

CORRELATION OF pH WITH RELATIVE TOXICITY OF SUPPOSITORIES

CODE NO. OF PREPARATION	RELATIVE EXTENT OF NECROSIS AND HEMORRHAGE	RELATIVE EXTENT OF EXUDATE	PER CENT OF ANIMALS SHOWING ONE-PLUS REACTION OR MORE IN EITHER CATEGORY	pH
ST3815	1	1	17	4.5
ST382	1.8	0.5	28	5.3
619	2.0	2	33	5.1
618-3	2.8	3	52	5.8
Mercurin	2.6	4	47	7.8
ST384	3.5	6	67	8.6
Salyrgan	3.8	7	62	8.9
S381, 618-2	3.6	12	75	8.6

It will be noted that of 37 cats receiving commercial salyrgan suppositories 62 per cent showed some type of reaction. The relative extent of damage to the gut wall was 3.8 times as great for salyrgan as for ST3815, the best of the preparations, and the extent of the exudative lesions was 7 times as great. Mercurin had an incidence of reactions of 47 per cent of the animals used. The extent of necrotic lesions was 2.6 times that produced by ST3815; the extent of exudative lesions, 4 times. It should be pointed out that, while the introduction of theophylline reduced the toxicity in varying degree, no preparation was found which, despite a low incidence of reactions, would not at some time cause a lesion in the colon of the cat.

Since it seemed that the pH of the medicinal constituent of the suppositories might be an important factor in determining toxicity, a portion of each suppository mass was extracted repeatedly with ether until free of cocoa butter, and the pH of a 2.5 per cent solution (or suspension) of the salt was measured accurately with the glass electrode. These values appear in column 5, Table IV.

TABLE V

MERCURY FOUND IN URINE AND LARGE INTESTINE TWENTY-FOUR HOURS AFTER ADMINISTRATION OF SUPPOSITORIES

CODE NO. OF PREPARATION	MG. Hg IN GUT	PER CENT UNABSORBED	MEAN PER CENT UNABSORBED	MG. Hg IN URINE	PER CENT EXCRETED	MEAN PER CENT EXCRETED
Mercurin	2.13	11.9		8.67	48.5	
	1.20	6.7		7.79	43.5	
	–	–		7.42	41.4	
	0.67	3.7		7.30	40.8	
	1.86	10.4		7.28	40.6	
	1.33	7.4		7.00	39.1	
	4.33	24.2		3.90	21.8	
	–	–	10.7	0.74	4.1	35.0
Mercurinic acid	11.4	57.0		3.75	18.8	
	10.1	50.0		2.81	14.0	
	4.29	21.4	40.7	2.45	12.2	15.0
ST3815	–	–		4.48	22.4	
	–	–		3.86	19.3	
	1.52	7.6		3.14	15.7	
	–	–		2.76	13.8	
	–	–		2.67	13.3	
	–	–		2.37	11.8	
	5.30	26.5		2.04	10.2	
	7.93	39.6	24.6	1.57	7.8	14.3
Salyrgan	4.56	25.9		4.34	24.6	
	–	–		3.72	21.9	
	6.79	38.6		3.58	20.7	
	–	–		3.51	19.9	
	7.78	44.1		3.05	17.3	
	7.50	42.6		3.04	17.3	
	3.04	17.3		2.81	16.0	
	6.80	38.6		2.10	11.9	
	–	–		2.07	11.8	
	8.98	51.0		1.93	11.0	
	7.76	44.0		1.32	7.5	
	7.88	44.8		1.24	7.1	
	8.06	45.8		1.14	6.5	
	6.18	35.1	38.9	0.98	5.6	14.2
ST384	–	–		7.10	35.5	
	–	–		3.13	15.6	
	–	–		3.01	15.0	
	4.35	21.7		1.19	5.9	
	–	–		0.64	3.2	
	–	–		0.51	2.5	
	–	–		0.35	1.8	
	–	–		0.14	0.7	10.0
ST3825	–	–		3.19	15.9	
	–	–		2.40	12.0	
	–	–		1.33	6.7	
	–	–		0.96	4.8	9.9
619	–	–		3.13	17.2	
	–	–		1.16	6.4	
	0.32	1.8		1.07	5.9	
	2.66	14.6		1.02	5.6	
	2.28	12.5		0.67	3.7	
	0.70	3.8		0.64	3.5	
	–	–		0.58	3.2	
	3.82	21.0	10.7	0.55	3.0	6.1
S381, 618-2	6.79	41.9		0.59	3.7	
	7.16	44.2		0.53	3.3	
	6.91	42.6	42.9	0.34	2.1	3.0

The correspondence of low pH values with lessened incidence and degree of reaction caused by various drugs is apparent. It can be seen, then, from Table IV that S381, the most toxic preparation, has nearly the highest pH, and that ST3815, the least toxic preparation, has the lowest pH. The two preparations that are available commercially, namely salyrgan and mercurin, show a definite relationship of toxicity to pH. Salyrgan with a pH of 8.9 has a toxicity almost as great as S381, whereas mercurin with a pH of 7.8 has a lower order of toxicity but is still more toxic than ST3815 with a pH of 4.5.

From the foregoing study of the local toxicity of mercurial diuretics when administered in the form of rectal suppositories, the following conclusions may be drawn:

1. All of the mercurial suppositories studied, when administered under the above-described conditions, may or may not produce pathologic changes in the large intestine of the cat, depending upon factors beyond our control at present.

2. No qualitative differences could be detected between the reactions produced by the drugs of various chemical compositions.

3. The local toxicity of mercurial suppositories may be compared by determining averages for the frequency and severity of the reactions produced in extended series of animals.

4. Apparently a distinct correlation exists between the pH of the drug in the suppository and its toxicity, preparations of low pH causing reactions least frequently. So far as local irritation is concerned, the chemical combination of theophylline with salyrgan or the admixture of salyrganic acid with salyrgan, or both, appears only to serve the purpose of reducing the pH of an aqueous solution of the suppository ingredients. It is, of course, impossible to say what theoretical connection this measured pH has with the chemical processes in the gut, but the correlation seems unmistakable.

In Table V are given the protocols for absorption from the gut and for the subsequent urinary excretion of mercury. From examination of the table the following may be concluded:

1. Absorption of mercurial diuretics, as studied by determining the mercury content of the large intestine of the cat twenty-four hours after introduction of a suppository, is highly irregular, and no correlation with local toxicity, pH, or chemical composition is apparent.

2. The excretion of mercurial diuretics, as studied by the mercury content of the urine collected during the first twenty-four hours after administration of a suppository, is also highly irregular. The values for the percentage excretion of mercury are grouped in Table V under the various preparations studied and are arranged in descending order of magnitude. In spite of the wide variations among data obtained with the same drug under conditions as nearly identical as possible, it is felt that a comparison of the means is of some value. Such a comparison shows (a) that mercurin is more rapidly excreted than any other preparation, and (b) that excretion following the administration of ST3815 is, on the average, as good as after commercial salyrgan and is better than after any of the salyrgan modifications which are of low pH and toxicity.

3. It is felt that the irregularity of absorption and excretion of mercurial diuretics after rectal administration is of significance in itself and would indicate that exact dosage by this route is impossible, even under optimum conditions.

4. It should be noted that no parallel can be drawn between the enhancing effect of theophylline upon the absorption of mercurial diuretics after intramuscular injection and its effect upon rectal absorption.

SUMMARY

A method for the experimental study of the rectal administration of mercurial diuretics has been suggested. A comparison has been made of the local toxicity, absorption, and elimination of suppositories of various chemical compositions. The lesions which they may produce in the colon of the cat have been described.

This investigation was carried out under the direction of Professor Arthur C. DeGraff, for whose guidance the authors wish to express their appreciation.

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LABORATORY METHODS

EVALUATION OF THE TUBERCLE BACILLUS CONCENTRATION METHODS OF PETROFF, POTTENGER, AND CHEMICAL FLOCCULATION*

A. H. Nagy, M.T., Sanatorium, Texas

IN THE examination of sputum for tubercle bacilli many workers have realized the futility of search in a straight smear of material containing comparatively few organisms. Consequently, various methods of concentrating the bacilli have been devised. The purpose of this study was to learn what might be expected with these concentration methods in routine clinical work in an average laboratory.

The methods studied were Petroff's, Pottenger's, and chemical flocculation. The respective techniques employed are as follows:

Petroff's Sodium Hydroxide Method (1)

1. Shake equal parts of sputum and 4 per cent sodium hydroxide for five minutes.
2. Incubate at 37° C. for thirty minutes or longer.
3. After complete homogenization centrifugalize at high speed.
4. Drain off supernatant fluid and neutralize sediment with normal hydrochloric acid.
5. Prepare smears from sediment, dry in air, and fix over flame.
6. Stain by Ziehl-Neelsen method.

Pottenger's Dilution-Flotation Method (2)

1. Shake equal parts of sputum and 0.5 per cent sodium hydroxide for ten minutes.
2. Digest in water bath at 56° C. for thirty minutes.
3. Add 1 ml. hydrocarbon (gasoline or xylene). Add about 200 ml. distilled water and shake ten minutes.
4. Allow hydrocarbon to collect at top (fifteen to twenty minutes).
5. The hydrocarbon layer is picked up in rubber bulbed pipettes and allowed to remain in vertical position until subnatant fluid separates from hydrocarbon (five to ten minutes). Slides of desired thickness are made from hydrocarbon and allowed to dry.

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Received for publication, November 8, 1938.

6. Wash slide in ether to remove hydrocarbon. Stain in carbolfuchsin three hours or longer. Decolorize with acid alcohol; do not allow more than thirty seconds as bacilli decolorize rapidly. If further decolorization is necessary, use 10 per cent sodium sulfate. Counterstain, preferably with 1 per cent aqueous picric acid.*

Chemical Flocculation Method (3)

1. Shake equal parts of sputum, and 4 per cent sodium hydroxide which contains 0.2 per cent $AlK(SO_4)_2$ (alum sulfate), and 0.002 per cent bromthymol blue.

2. Digest in water bath at 37° C. for thirty minutes and shake for ten minutes.

3. Add N/2.5 hydrochloric acid until neutral. Shake thoroughly. If flocculation does not occur in less than five minutes, add 0.2 ml. 1 per cent $FeCl_3$ (ferric chloride) and shake.

4. Centrifugalize at high speed to pack precipitate.

5. Prepare smears from sediment, dry in air, fix over flame, and stain by method desired.

Since it is the purpose of any concentration method to collect the greatest number of bacilli in the smallest possible volume, the first series of tests on 50 positive sputa was made to compare the number of bacilli which could be collected from 15 ml. samples of sputum sodium hydroxide digest by Petroff's method centrifugalized at 3500 r.p.m. for thirty minutes; by Pottenger's method and by chemical flocculation method centrifugalized at 1750 r.p.m. for five minutes and compared to direct smear of "choice particles" of the original sputum sample. The use of the 15 ml. sputum digest quantity was chosen for the convenient use of the 15 ml. conical centrifuge tube.

TABLE I*

METHOD	AVERAGE NUMBER BACILLI PER FIELD	CONCENTRATION FACTOR
Straight smear of choice particles	3.3	× 1
Petroff's at 3500 r.p.m.—30 min. centrifugation	19.8	× 6
Pottenger's dilution-flotation	97.6	×29
Chemical flocculation at 1750 r.p.m.—5 min. centrifugation	64.2	×19

*Mechanically homogenized. Fifty microscopic fields examined.

Following Hanks'[3] example, direct examination was used as unity to arrive at a figure from which the concentration factor was computed. Since it is evident that the advantage of distribution of bacilli is that the chance of finding a clump of 5, 10, or 20 bacilli represents one chance, all clumps of bacilli were counted as "1"; if the clump is broken up, the chance of diagnosis is increased 5, 10, or 20 times.[2] As pointed out by Hanks,[3] the digesting process doubles the volume of sputum sample, and unless the breaking-up of clumps of bacilli is sufficient to double the possibility of finding them, the concentration factor must

*In step 1, a 4 per cent sodium hydroxide was used in place of 0.5 per cent in order that all sputum-digest samples for the various tests may be uniform. This increase in the per cent of sodium hydroxide used necessitated the lowering of incubation temperature in step 2 to 37° C., or otherwise the bacilli decolorized quite readily. Methylene blue was used as a counterstain.

be divided in two in order to indicate the actual advantage of concentration. This point was later proved as shown by Table II, assuming that hand shaking is inferior to mechanical shaking to bring about complete homogenization.

TABLE II*

METHOD	AVERAGE NUMBER BACILLI PER FIELD HOMOGENIZED		AVERAGE NUMBER BACILLI PER FIELD OCCASIONAL SHAKING	
Straight smear of choice particles	3.3	× 1′	3.3	× 1′
Petroff's at 3500 r.p.m.—30 min. centrifugation	19.8	× 6′	14.2	× 4′
Pottenger's dilution-flotation	97.6	×29′	47.9	×15′
Chemical flocculation at 1750 r.p.m.—5 min. centrifugation	54.2	×19′	43.1	×13′

*Fifty microscopic fields examined.
'Concentration factor.

A second series of tests was carried out on 25 sputa which were known to be repeatedly negative by straight smear examination but positive on some previous examination. Table III shows the comparative results of the respective concentration methods.

TABLE III*

METHOD	AVERAGE NUMBER BACILLI PER 100 FIELDS	CONCENTRATION FACTOR
Straight smear of choice particles	0.4	× 1
Petroff's at 3500 r.p.m.—30 min. contrifugation	2.9	× 7
Pottenger's dilution-flotation	13.1	×32
Chemical flocculation at 1750 r.p.m.—5 min. centrifugation	7.4	×18

*Mechanically homogenized. Two hundred microscopic fields examined.

A third series of tests was performed as in the first, except that the quantity of sputum digest was not limited to 15 ml. This was undertaken to ascertain the value of the use of larger quantities of sputa. In Petroff's and the chemical flocculation methods this was found unsatisfactory, because the concentrated sediment proportionally increased in volume sometimes to the extent that only a small portion could be considered for routine use. This objection, however, was not encountered in Pottenger's method. In one instance as much as 150 ml. of the sputum digest were used without an increase in the amount of hydrocarbon necessary to collect the bacilli. Table IV shows the results of this series of tests.

TABLE IV*

METHOD	AVERAGE NUMBER BACILLI PER FIELD†		AVERAGE NUMBER BACILLI PER FIELD‡	
Straight smear of choice particles	4.1	× 1′	4.1	× 1′
Petroff's at 3500 r.p.m.—30 min. centrifugation	22.6	× 6′	23.5	× 6′
Pottenger's dilution-flotation	112.7	×28′	163.8	×40′
Chemical flocculation at 1750 r.p.m.—5 min. centrifugation	64.8	×16′	68.4	×17′

*25 microscopic fields examined.
†15 ml. sputum digest used.
‡Any greater volume of sputum digest used.
'Concentration factor.

It is interesting to note that in the previous series of tests the concentration factors of the respective methods remained comparatively the same, but in this series of tests the volume of sputum digest was increased; in Petroff's and the chemical flocculation methods there was a slight decrease in the concentration factor, whereas a great increase was found by the Pottenger's method. This increase of the concentration factor in Pottenger's method was persistent throughout this series of tests. On one specimen, of frothy, tenacious, mucopurulent consistency, 150 ml. of sputum digest were used. The findings were as follows: Straight smear, 50 bacilli in 50 fields, or an average of one bacilli in a field. Pottenger's concentration of the same specimen was estimated to contain 300 bacilli to a field, or a concentration factor of 300. This figure was not considered in Table IV or in the evaluation of the methods.

From the foregoing tables we derive the following concentration factors: Petroff's—sodium hydroxide method—×6; chemical flocculation method—×18. The concentration factors of Petroff's and the chemical flocculation method closely resemble the findings of Hanks[3] in his comparison of the two methods. The concentration factor in Pottenger's method of ×33 in the series of tests with 15 ml. sputum digest volume and of ×40 in the series using greater volumes of sputum digest is considerably under ×60, the concentration factor derived by Pottenger.[2] However, his method of computing the concentration factor was based on the number of bacilli found in a unit length of time, whereas the method used in this series of tests was based on the number of bacilli found per field regardless of the time consumed in the examination. It is a fact that the average time required to count the bacilli in 50 fields from preparations of straight smears, Petroff's or chemical flocculation, was as much as 200 per cent greater than the time required to count a like number of fields in preparations from Pottenger's method.

DISCUSSION

The criteria for judging the relative efficiency of concentration methods evaluated were based chiefly on two principles: First, the superior method must be efficient and accurate, without fail, in a reasonable length of time. Second, the technique must be simple; that is, complicated, special apparatus, unstable chemicals, and much handling must be avoided. With these points and the results of the previously related series of tests, Petroff's and the chemical flocculation methods are considered not sufficiently efficient to warrant further discussion even though their simplicity and the required length of time to perform the tests are commendable.

The superiority of Pottenger's dilution-flotation method is enhanced by its simplicity and the applicability of the method to large or small volumes of work. The apparatus which we found preferable are as follows: Phosphorus determination flask, 450 ml.—this type of flask has straight walls and a neck of 22 mm. diameter in which the hydrocarbon layer readily collects, and is sufficiently large to permit easy and thorough cleaning. Furthermore, any number of these flasks may be secured in an easily constructed wooden frame and clasped into a Kahn shaking machine for the homogenization process. The final hydrocarbon-layer separation step is carried out in Kahn serum transfer

pipettes set vertically in test tubes in a rack. Subnatant fluids from these pipettes are easily discarded and slide preparations are made of desired thickness by discharging the remainder of the bacillus-collecting hydrocarbon layer. Air - or oven-drying may be used. The slide preparations do not peel during the process of staining as so often happens in preparations from other methods. Under the microscope a maximum penetration of stain is evident. There is an absence of grit and other residues. The background is homogeneous, permitting a diffuse light. The bacilli are evenly distributed and readily recognized.

CONCLUSIONS

Pottenger's dilution-flotation method of concentration gives an average of 33 times as many bacilli as can be found in direct smears of choice particles in routine work. The efficiency of this method is twice that of chemical flocculation and four times that of Petroff's. According to Pottenger,[2] this is as accurate as guinea pig inoculation and can detect the presence of as few as 1,000 bacilli in twenty-four-hour collections of sputa, whereas to find tubercle bacilli in a straight smear by microscopic examination requires the presence of 100,000 tubercle bacilli per 1 ml. of sputum.[4]

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ROUTINE LABORATORY EXAMINATIONS FOR C. DIPHTHERIAE*

C. Alfred Perry, Sc.D., and Elizabeth Petran, Ph.D.
Baltimore, Md.

ROUTINE examinations for *C. diphtheriae* have been made for so many years that one is likely to assume that this is one of the simpler bacteriologic examinations which anyone with moderate training is qualified to do, whereas in reality it is one calling for seasoned judgment and discrimination. Several years ago an error of approximately 75 per cent was found in the results obtained in one laboratory. The source of error in this case was due to placing entirely too much reliance on cellular morphology and granules. Many diphtheroids when stained for granules are indistinguishable from *C. diphtheriae*, while many strains of *C. diphtheriae* lack granules. So certain was the bacteriologist that he knew *C. diphtheriae* when he saw it that he could be convinced of his error only after careful and painstaking pure culture studies had definitely established the identity of the organism.

*From the Bureau of Bacteriology, Maryland State Department of Health, Baltimore.
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Many weak points in the methods routinely used in testing for *C. diphtheriae* were recognized. During the past six or seven years a number of studies have been carried out which we believe have greatly improved the quality of our routine work.

LIMITATIONS OF LÖFFLER'S MEDIUM

One of the first steps in these studies was to determine the efficiency of Löffler's medium for the growth of *C. diphtheriae*. To our surprise, the quality of nearly every batch of Löffler's medium for growth and cellular morphology varied tremendously. In many instances abundant growth could be obtained, but cellular morphology was so unsatisfactory that the correct identification of various strains of *C. diphtheriae* would often be impossible. With other batches the amount of growth would be so scanty that overgrowth of *C. diphtheriae* by other throat and nose bacteria would be almost certain to occur even though in such instances cellular morphology was very satisfactory. Many months were spent in trying out different methods for the preparation of a Löffler's medium which would give reasonably good growth and satisfactory cellular morphology. The time and temperature of autoclaving and the pH of the medium were found to be the chief factors influencing growth and cellular morphology. The following method has been found to give uniformly good results.

Clear beef or hog serum	75 per cent
Beef infusion broth pH 7.6	25 per cent
Dextrose	0.25 per cent

Dissolve the dextrose in the infusion broth and add to the serum. Adjust to pH 8.3 with sodium hydroxide. Tube in 4 to 5 c.c. quantities. Place in the autoclave in a slanting position. Close the door and fasten tightly. Close the air valve to keep all air in before turning on the heat. Autoclave one hour at 15 pounds, then one and one-half hours at 20 pounds (with air retained). Final pH will be 7.6 to 7.8.

It is our practice to give every batch of Löffler's medium a number and to carefully test each for growth and cellular morphology with select strains of *C. diphtheriae*. No batch is released for routine use until it has been so tested and approved. A check in one laboratory where the Löffler's medium was found to give very poor growth but satisfactory cellular morphology revealed that this laboratory had found only about half as many positive cultures from specimens sent in for diagnosis, release, or carrier as in some others. The implication that the poor growth-promoting quality of the medium was responsible seemed justified. It was also obvious that many errors were due to failure to correctly identify *C. diphtheriae* when identification depended entirely on microscopic appearance (Table I). These limitations of Löffler's medium directed our attention to other methods.

ADVANTAGES OF DOUGLAS' POTASSIUM TELLURITE MEDIUM

Our interest was directed to Gilbert's modification[1] of Douglas' potassium tellurite medium* because of the success others had had with this medium.

*Dissolve 1.5 per cent of granulated agar in beef infusion broth. Adjust pH to 7.2 to 7.4, bottle in convenient amounts without filtration, and sterilize in the autoclave at 15 pounds for twenty minutes. Melt this base in an Arnold sterilizer when ready to use and cool to 50° C. Add 5.0 per cent sterile human or hog serum, 0.2 per cent of dextrose from a sterile solution (2 c.c. of a 10 per cent dextrose solution to 100 c.c. of base) and 0.01 per cent of potassium tellurite (1 c.c. of a 1 per cent sterile solution). Distribute in Petri dishes.

After using it routinely for four years and after trying McLeod's chocolate tellurite[2] and Clauberg's laked blood tellurite medium,[3] we found it the most satisfactory.* It is easier to prepare, and all strains of *C. diphtheriae* grow well on it though colonies are much smaller than those on Clauberg's medium. It has the advantage of transparency.

The chief difficulty with this medium, as with all other media containing potassium tellurite, is controlling the concentration of tellurite. This may be accomplished by using a solution free from precipitate. We have found that potassium tellurite begins to precipitate from solution when the pH is lower than 9.0.† We, therefore, adjust the pH of a stock solution made according to the directions of Ruth Gilbert to pH 9.5 with 10 per cent potassium hydroxide. In filtering through a Seitz filter a precipitate forms in the first portion due to a change in pH by the filter pad. This portion should be refiltered by adding it to the unfiltered solution. In this way we have been able to obtain a crystal clear solution. If this is kept tightly stoppered and is withdrawn only with chemically clean pipettes, it will remain free of precipitated potassium tellurite. We have found this precipitate to be responsible for wide fluctuations in growth of *C. diphtheriae* in Douglas' medium because it varies the concentration of tellurite in the medium.

Another factor responsible for failure to find colonies of *C. diphtheriae* on tellurite plates is overgrowth by other bacteria, mostly the staphylococci. We have found that when the medium gives optimum growth of *C. diphtheriae,* overgrowth is not likely to occur since the colonies of *C. diphtheriae* may be seen protruding through the mass growth of staphylococci. When a very small number of colonies of *C. diphtheriae* are present, no doubt overgrowth does occur.

Our experience with Douglas' tellurite medium has been, briefly, that *C. diphtheriae* is frequently found when absent or unrecognized on Löffler's medium. However, when the tellurite medium is used in conjunction with Löffler's our experience has been that the examiner soon begins to recognize certain types as *C. diphtheriae* that he would not if the tellurite medium had not been used. Thus in 1934, 10.9 per cent of positive incidences were found on tellurite medium which were negative on Löffler's, while in 1935, 1936, and 1937, the percentages were much less (Table II). During 1936 many more positive results were found on Löffler's than on tellurite medium. This was due to the difficulty of obtaining the correct concentration of tellurite as indicated above. The use of both media obviated doubtful reports to physicians after 1934. The definite identification of *C. diphtheriae,* of course, is of great value in correct diagnosis by the physician and in the avoidance of unnecessary detention of cases and carriers.

The great value of tellurite plates for the accurate and dependable identification of *C. diphtheriae* is beyond question. Even with every batch of Löffler's

*An ideal medium, however, for culturing *C. diphtheriae* from throat and nose swabs has not yet been developed. Further study of this problem is being made.

†Potassium tellurite probably precipitates from aqueous solution in the following way:

$$K_2TeO_3 + HOH \longrightarrow TeO_2 \downarrow + 2KOH$$
$$K_2TeO_3 + CO_2 \longrightarrow TeO_2 \downarrow + K_2CO_3$$
$$TeO_2 + 2KOH \longrightarrow K_2TeO_3 + H_2O$$

Therefore, in an excess of OH ions and with CO_2 ions excluded by use of a tight rubber stopper, precipitation of TeO_2 can be prevented. Complete solution, of course, insures a uniform concentration of potassium tellurite, and if medium and solution are carefully measured, the concentration of potassium tellurite will be constant in all batches of medium.

TABLE I

COMPARATIVE RESULTS BY DIFFERENT EXPERIENCED BACTERIOLOGISTS BASED ON EXAMINATIONS OF SMEARS FROM LÖFFLER'S MEDIUM ONLY

BACTERIOLOGIST	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
N. P. S.	−	+	−	−	−	−	−	+	−	+		−	−			S	+	
O. R. W.	+	+	−	+	−	−	+	+	+	+	−	+	−	+	−	+	+	+
C. A. E.	S	+	S	−	S	−	−	+	−	+	−	−	−	+	−	−	−	+
C. A. P.	+	+	−	+	−	−	+	+	+			+	−	+				+
R. H. M.	S	+	S	−	−	S	−	+	S	+	−	S	−	+	+	S	S	+
A. A. H.	+	+	−	−	−	−	−	+	−	+	−	−	−	+	−	+	+	+
M. W.	+	+	+	−	−	−	−	+	−	+	−	S	−	S	−	−	−	−
T. F. D.	+	+	+	−	−	S	+	+	S	+	−	+	−	S	−	−	−	−

S = suspicious.

NOTE: Preparations selected because of difficulty in decision as to whether or not they were diphtheria.

TABLE II

COMPARISON OF NUMBER AND PER CENT OF POSITIVE CULTURES OF C. DIPHTHERIAE ON LÖFFLER'S AND DOUGLAS' MEDIA

YEAR	LÖFFLER + TELLURITE +		LÖFFLER + TELLURITE − OR UNS.		LÖFFLER − OR UNS. TELLURITE +		TOTAL POSITIVES
	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	
1934*	295	80.6	9	2.5	62	17.0	366
1935†	492	91.3	25	4.6	22	4.1	539
1936*	218	80.8	32	11.8	20	7.4	270
1937†	1036	88.5	72	6.1	69	5.9	1177

*Central laboratory.

†Central and branch laboratories.

Uns. = unsatisfactory.

medium carefully controlled, it is never possible to be certain of many results if only Löffler's medium is used. No bacteriologist in our service who has once become accustomed to the use of tellurite plates has felt that he could again depend solely on Löffler's. It is our practice to read all tellurite plates after twenty-two to twenty-four hours' incubation at 37° C. and to pick suspicious and typical colonies to microslides. These are stained with Löffler's methylene blue. It is usually a simple matter to identify the various types and strains of *C. diphtheriae* in these pure or almost pure cultures from tellurite plates after one becomes familiar with the cellular morphology of *C. diphtheriae* on this medium. We have found[4] that an experienced person can identify cultures of *C. diphtheriae* as *gravis*, *mitis*, or *intermediate* types with a high degree of accuracy, from cellular morphology on tellurite plates. The use of tellurite plates greatly facilitates virulence tests when these are necessary. Colonies may be picked without delay from primary plates, and there is not the chance of picking several colonies which may be descendants of one organism as when the growth from a Löffler slant is plated. Through the use of tellurite plates one is unquestionably enabled to recognize *C. diphtheriae* on Löffler's with far greater accuracy than is otherwise possible.

We find, however, a considerable percentage of Löffler slant cultures positive when tellurite plates are negative (Table II). It is our practice to examine all cultures for diagnosis or release on both media. However, where we have large numbers of throat and nose swabs from groups of school children and others for detection of carriers, we usually use either tellurite plates or Löffler slants, but not both, due to the work involved and the doubtful importance of carriers, with very few diphtheria organisms, in the spread of the disease. With proper controls tellurite plates are not believed to be negative except rarely when very small numbers of *C. diphtheriae* are present in the throat or nose. Not more than one specimen should be inoculated onto a single plate except in emergencies. Obviously, specimens from different persons should never be inoculated on one plate.

Douglas' tellurite medium is prepared as slants and is used in containers for mailing specimens to the laboratory. These slants are sealed with paraffined cork stoppers and carefully tested for sterility. The tellurite medium has been found to inhibit spore formers and spreaders, but in the strength used has not been found to prevent the growth of either *C. diphtheriae*, hemolytic streptococci, or pneumococci. Oftentimes the tellurite slants are positive for *C. diphtheriae* as received. We believe the medium is superior to Löffler slants for this purpose.

VALUE OF DIRECT SMEARS IN DIAGNOSIS OF DIPHTHERIA

When diphtheria bacilli can be identified by direct microscopic examination of stained preparations of material from the throat or nose lesions, this is the method of choice, for it permits an immediate report. Frequently the organisms are as distinctive in such direct smears as in cultures. At times they occur in large numbers. After several years' experience with direct smears, bacteriologists both in our central and branch laboratories have been able to find positives in an increasing percentage of cases (Table III). Cultures are always made

TABLE III

VALUE OF DIRECT EXAMINATIONS FOR C. DIPHTHERIAE

	1933	1934	1935	1936	1937
Number of positive cases with satisfactory direct smears	221	175	211	170	187
Number of positive cases on direct smear	62	98	90	91	132
Per cent positive	28.0	56.0	44.8	53.5	70.7
Number of errors, direct smear positive, culture negative	4	6	6	0	1
Per cent of error	1.8	3.4	2.8	0	0.5

NOTE: Only those specimens for diagnosis on which a satisfactory direct smear was made and on which the cultures subsequently yielded *C. diphtheriae* are considered in this tabulation.

regardless of the finding of *C. diphtheriae* in direct smears. During the past two years only one direct smear was considered positive which did not confirm on culture. In many instances only scanty material was obtained on the swabs. Undoubtedly, a very large percentage would be positive in this way if physicians would use care to secure part of the membrane on the swab. By emphasis on this point we hope to increase still further the percentage of positive direct smears.

In the cases with positive direct smears the physician is given this information by telephone as a preliminary report. A written report based on culture is sent later. All preparations, whether direct smears or cultures, are stained with Löffler's methylene blue. No other stain has been found as satisfactory. On several occasions preparations have been found which were stained purplish rather than blue, and in one instance at least, a large error was the result of incorrect staining. On investigation it was found that too much alkali had been added to the stain. It is important that the final pH of the methylene blue be approximately 6.0.

INCIDENCE OF NASAL DIPHTHERIA

It is a common practice in taking cultures for the diagnosis, release, or detection of diphtheria carriers to take material only from the throat. While the fallacy of such a practice is obvious, a full appreciation of the matter does not always exist. One of us was a carrier of a nonvirulent strain of *C. diphtheriae* for several years and being subject to frequent attacks of sinusitis, he made repeated cultures of the discharge from the nose. The danger from any one with discharging sinuses who happened to be a carrier of a virulent strain of *C. diphtheriae* was obvious from the fact that enormous numbers of *C. diphtheriae* were frequently found during such attacks. Since the hands are perhaps more frequently soiled with discharges from the nose than from the throat, particularly during colds when such contamination may be very great, it is apparent that people with positive nose cultures are most likely to spread the disease. The danger from carriers is probably not during the time when they harbor a few virulent diphtheria bacilli, but during the time when such carriers develop a cold and virulent diphtheria organisms are present in enormous numbers. In order to have some quantitative data on the incidence of nasal infections, the percentage of positive nose cultures was compiled for three years. The percentage of cases with both throat and nose positive was 18.5, 19.0, and

26.1, respectively. The percentages of cases with positive nose and negative throat cultures were 13.8, 12.0, and 2.5. The total percentages for cases with positive throat or nose cultures were, therefore, 32.3, 31.0, and 28.6. Almost a third of all persons carrying *C. diphtheriae* carried this organism in the nose (Table IV).

TABLE IV

INCIDENCE OF NASAL INFECTION OF DIPHTHERIA

	1933		1934		1935	
	NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT
Total positives (nose and throat)	563	100.0	782	100.0	241	100.0
Nose and throat positive	105	18.5	150	19.0	63	26.1
Nose positive, throat negative	76	13.8	96	12.0	6	2.5
Nose negative, throat positive	380	67.8	536	68.5	172	71.4

DISCUSSION AND SUMMARY

Though it is generally accepted that Löffler's medium is the one of choice for routine culturing of *C. diphtheriae* from throat and nose swabs, the growth and cellular morphology of various strains of *C. diphtheriae* on different batches of this medium have been found so variable that errors as high as 50 to 75 per cent have occurred at times. Unless Löffler's medium is very carefuly prepared with particular attention to pH, time, pressure, and air conditions during autoclaving, satisfactory batches of this medium cannot be obtained. Every batch should be tested for final pH and for growth and cellular morphology of select strains of *C. diphtheriae*. Even with such care, Löffler's medium is not entirely satisfactory, for overgrowth by other bacteria often occurs and at times examinations are impossible due to liquefaction. Even when the medium is adjusted to insure optimum growth and cellular morphology for *C. diphtheriae*, the identification of this organism on Löffler's medium alone cannot, in many instances, be made with certainty. This makes it necessary for the laboratory to give suspicious or doubtful reports which are unsatisfactory both to physicians and health officers.

Serum tellurite agar plates have been used routinely in the laboratories of the Maryland State Department of Health for five years. These have been of such value that none of the more than a dozen bacteriologists who have become familiar with the use of this medium would want to depend solely on Löffler's medium. The most important result from the use of tellurite plates has been the elimination of all suspicious or doubtful reports. Nervous strain, due to the tension of trying to decide if certain *C. diphtheriae*-like bacilli are really diphtheria bacilli or diphtheroids, is eliminated. Colonies of *C. diphtheriae* can usually be identified by a simple inspection of the plates, though colonies should always be transferred to slides for a microscopic check. The cellular morphology of *C. diphtheriae* on tellurite medium, while different from that on Löffler's medium, is very characteristic. Not only can *C. diphtheriae* be easily differentiated from the diphtheroids but with experience those interested can learn to identify the types (*gravis*, *mitis*, and *intermediate*) if necessary, with a high degree of accuracy.

The tellurite medium described has also been found to be superior (in slant form) to Löffler's medium for use in specimen mailing outfits. It is easily prepared, adheres more tenaciously to the tubes, is not easily broken up, and is more resistant to contamination. It suppresses growth of liquefying spore-formers and many other bacteria, but permits the growth of *C. diphtheriae*, hemolytic streptococci, and pneumococci.

The accuracy of the direct smear in the laboratory diagnosis of diphtheria has been carefully studied. During the last three years over half the swabs from cases of diphtheria have been positive by direct smear. During the past two years, only one direct smear reported as positive has failed to be confirmed. In many of these smears there was insufficient material for a proper examination. If the cooperation of physicians can be secured to get part of the necrotic tissue from the lesion on the swab, there is little question that positive results could be obtained in most instances. The direct smear in the hands of experienced workers is of utmost value.

The incidence of nasal infections has been found very high. Nearly one-third of all cases had nose infections. The danger from spreading diphtheria is probably greater in children and adults with nose infections than in those with the site of infection only in the throat. This danger may be paramount when the individual develops a cold. Routine cultures of the nose are just as important as cultures of the throat.

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A NEW AND MORE PRACTICAL CULTURE TUBE DESIGNED FOR MORE RAPID DIAGNOSIS*

IRVING S. BARKSDALE, M.D., F.A.P.H.A., GREENVILLE, S. C.

THE wise and time-honored teaching for many years current in our American medical colleges—"If in doubt, give diphtheria antitoxin!"—has been the result of the slower methods of culturing, growing, and recognizing by direct microscopic examination of the stained smear the Klebs-Löffler bacillus from the nose and throat of suspicious cases. The use of the customary Löffler coagulated beef-blood slant has necessitated the lapse of at least twenty-four hours before the clinician could have the report from the bacteriologist, and nearly always such a delay without antitoxin in positive cases is decidedly against the patient's prompt recovery from diphtheria and places the heart and other important organs in immediate jeopardy from the effects of the circulating, soluble diphtheria toxin which acts so quickly to do its irreparable damage.

With the classic Löffler slant now in common use, a lapse of fully twenty-four hours or longer was necessary for the bacilli to multiply in sufficient numbers to be recognized by the usual staining and microscopy, because in this type of tube, there is usually quite a large area of coagulated beef-blood slant and condensed moisture to be occupied by the organisms for ready and accurate recognition in the finished stained specimen. Such a long time was necessary between the taking of the nose and throat culture by the clinician and the laboratory report by the bacteriologist because of one hindering factor—a small amount of nose or throat organisms on a small sterile swab in a large amount of culture media and a sufficient amount of time allowed for the organisms to multiply in sufficient quantities for the bacteriologic examination. Possibly a second delaying or hindering factor is often present in the stock culture tubes whether they be kept carefully in the laboratory under refrigeration or carelessly in the drug store on a counter or hot, dusty shelf, and that factor is inadequate or absence of moisture in the tube, which is such a common occurrence when the attempt is made by the manufacturer to close the culture tube with the usual paraffined cork.

Ever bearing in mind these delays in and hindrances to bacterial growth, at the same time surmising any and all difficulties that might arise from physicians attempting to wait for the bacteriologic verdict, and ever bearing in mind the dangers of the culture tube without sufficient moisture for bacterial growth, we dared to depart from the use of the time-honored or classic Löffler slant and to change the method of making and preserving these culture tubes rather radically. The amount of uncoagulated beef-blood serum in the classic Löffler slant was reduced from 5 to 8 c.c. in a medium-sized test tube to 1 c.c. or less in a

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very small container. Instead of using the customary small and rather tightly wrapped cotton-tipped applicator, we began to use a much larger and less tightly wrapped combination. Following these somewhat drastic changes we have a large amount of nose and throat secretion on a larger and more loosely wrapped cotton tipped applicator with which to inoculate a relatively much smaller amount of culture media sealed with fresh, tightly fitting rubber corks, the evaporation from which is almost nihil. Instead of inoculating the slant with the applicator and discarding the applicator, it was retained by breaking off the stick near the cotton tip and allowing it to remain in the water of condensation at the bottom of the slant when the culture tube is held in the upright position. Such inclusion of the original applicator insures the inoculation of the slant with all of the bacteria on the tip of the applicator. Using this relatively small volume of culture media and large amount of nose and throat secretions instead of the large volume of culture media and small amount of nose and throat secretions as in the old classic Löffler culture tube, the bacteriologic laboratory is enabled to report to the clinician the microscopic examination of the stained slide in a very short time, after three to four hours' incubation at body temperature, 37.6° C.

Method of Preparation of Culture Tube.—1. The Löffler coagulated beef-blood medium (Difco) is prepared in accordance with instructions on the label of the dehydrated product of the Digestive Ferments Co., with one exception—1 c.c. instead of 5 c.c. is placed in a very small rubber stoppered tube.

2. One-half dram homeopathic vials or even smaller containers are used, slanted to the desired angle and sterilized in accordance with directions on the label of dehydrated beef-blood medium.

3. Soft, fresh, pliable, and tightly fitting rubber stoppers are first sterilized in 70 per cent alcohol for fifteen minutes, and then placed in the autoclave with large ends of the stoppers up for sterilization with the culture media.

4. The little vials must be stoppered immediately to prevent the loss of the water of condensation in them. If only a small amount of water of condensation remains, a few drops of sterile water must be added before stoppering.

5. Store with or without refrigeration, tightly stoppered.

Method of Taking the Culture, Incubation, and Practical Advantages of the Vial Löffler Culture Tube.—1. Nose, throat, and other cultures (as this medium can be used for general purposes) are taken in the usual manner.

2. The end of the culture swab is broken off short and left in the bottle to conserve all bacteria from the original culture.

3. The rubber stopper is then placed in tightly to prevent loss of moisture.

4. Incubate for three to four hours at 37.6° C. After this time there are large numbers of bacteria in sufficient quantities for the diagnosis to be made from the stained slide microscopically.

SUMMARY

1. The disadvantages of the conventional, old type Löffler coagulated beef-blood slant have been put forth, the principal disadvantage being due to the length of time necessary between the taking of the culture by the clinician and the final report of the bacteriologist.

2. The principal advantages of the new vial type Löffler culture tube are due to a relatively large amount of nose and throat secretion inoculated into a small amount of culture media, the bacteria of which have multiplied in sufficient numbers to enable accurate bacteriologic diagnosis in from three to four hours; the necessity of conserved moisture of condensation in the tube has also been discussed.

3. The method of preparation of the culture tube has also been given in detail, and certain technical precautions have been mentioned.

4. The method of taking the culture, incubation, storage and practical advantages of the vial Löffler coagulated beef-blood slant have been described in detail.

I am very grateful to Frank P. Simpson, Aileen Carlisle, Gladys K. Mullenix, R. N., L. Bates Fuster, R. N., Florence L. Deadwyler, and a number of our local physicians for their technical assistance in the introduction and use of the vial Löffler culture tube for the more rapid bacteriologic diagnosis of certain infections.

OBSERVATIONS ON MAC CONKEY'S AND DESOXYCHOLATE-CITRATE AGARS FOR THE ISOLATION OF DYSENTERY BACILLI*

J. V. Irons, Sc.D., S. W. Bohls, M.D., Thelma DeShazo, B.S., and L. L. Hewlett, B.A., Austin, Texas

IN RECENT years culture media appear to have been more improved for the isolation of typhoid than of dysentery bacilli. Leifson[3] devised desoxycholate-citrate agar for the isolation of Flexner and Schmitz dysentery bacilli. Paulson[4] reported that plain desoxycholate and desoxycholate-citrate agars were superior to Endo's and eosin-methylene blue agars for the investigation of infectious bowel disorders. Hardy and Watt[1] have confirmed these observations, particularly as they applied to the isolation of Flexner bacilli. Recent modifications of Wilson and Blair's medium by Tabet[5] have failed to render this medium satisfactory for growth of dysentery bacilli. Jones[2] found brilliant green-eosin agar quite inferior to MacConkey's agar for the isolation of dysentery bacilli. Our purpose is to record some experiences with and observations on the comparative value of MacConkey's and desoxycholate-citrate agars for the isolation of dysentery bacilli from stools in cases of acute diarrheal disorders.

PROCEDURE

Fecal specimens unpreserved or diluted in 30 per cent glycerol saline solution were seeded on plates of the two media. Although on occasion we have prepared our own media, we have generally relied upon the dehydrated products bearing Difco or BBL labels.† Usually two plates of each medium were em-

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†These products were obtained from the Difco Laboratories, Detroit, and the Baltimore Biological Co., Baltimore.

TABLE I

GROWTH ON DESOXYCHOLATE-CITRATE AGAR

ORGANISM	DESCRIPTION OF GROWTH IN TWENTY-FOUR TO FORTY-EIGHT HOURS	
	LEIFSON	IRONS AND ASSOCIATES
B. coli	Usually considerably inhibited; colonies slowly turned red	Usually strongly inhibited; colonies slowly turned pink or red
Aerogenes	Usually considerably inhibited; mucoid colonies with pink centers and colorless peripheries	Usually considerably inhibited; mucoid colonies slowly turning pink or red, especially at center
Enterococci	No growth	No growth
Alkaligenes	Usually no growth, or strongly inhibited	Greatly inhibited, or very small colorless colonies
Pyocyaneus	Large slightly grayish-green colonies	Large grayish or olive-colored colonies
Proteus	Smooth, nonspreading, colorless colonies; some strains slightly inhibited	Smooth, nonspreading, nearly colorless colonies
Yeast and molds	Many grew well	Slight inhibition of some; many grew luxuriantly
B. morgani	Complete inhibition	No growth, or very small colorless colonies
Typhoid	Fairly large, translucent, colorless colonies; some old strains inhibited	No inhibition of recently isolated strains; translucent, colorless colonies
Paratyphoid	Large, colorless, translucent or opaque colonies; data incomplete	No recently isolated strains tested; stock cultures varied
Dysentery (Flexner)	Fairly large, colorless, opaque colonies	All recently isolated strains have grown well; colorless opaque colonies
Dysentery (Alkalescens)	No growth	Marked inhibition
Dysentery (Shiga)	Stock cultures did not grow	Stock cultures were suppressed
Dysentery (Duval-Sonne)	Poor growth	Some recently isolated strains grew poorly
Dysentery (Schmitz)	Good growth	A stock culture was suppressed

ployed per specimen. A much larger inoculum was permissible on desoxycholate-citrate than on MacConkey's plates. Suspicious colonies were subcultured on Russell's and other differential media. Fermentation of rhamnose and dulcitol was helpful, that of the former for early separation of Duval-Sonne bacilli and that of the latter for early separation of alkalescens bacilli, from the Flexner group. In every instance, however, final identification rested upon specific agglutination tests.

EXPERIMENTAL

On MacConkey's agar most enteric organisms grow luxuriantly. Since nonlactose fermenters grow much alike, differentiation by inspection is relatively impractical. Most nonlactose fermenters grow readily in colorless or

TABLE II

COMPARATIVE RESULTS ON SELECTED STOOLS CULTURED ON MACCONKEY'S AND DESOXYCHOLATE-CITRATE AGARS

NO.	SOURCE	MAC CONKEY'S	DESOXYCHOLATE-CITRATE
285	Bloody dysentery	Numerous colon and 5 or 6 Flexner colonies	A few colonies of aerogenes and numerous colonies of Flexner bacilli
286	Bloody dysentery	Numerous colon and 3 or 4 Flexner colonies	Many small lactose fermenters and about 50 Flexner colonies
117	Sudden diarrhea	Numerous colon colonies; nonlactose-fermenting colonies not found on 3 plates	Ten or 12 Flexner colonies and a few colonies of aerogenes
851	Bloody dysentery	Numerous Flexner and colon colonies	Numerous Flexner, aerogenes, and pin-point colonies
904	Bloody dysentery	Numerous colon, many proteus and alkaligenes colonies	Numerous proteus, pin-point and unidentified opaque colorless colonies
642	Sudden diarrhea	Numerous colon, alkaligenes, and several unidentified colonies	Numerous pin-point and unidentified colorless colonies
966	Sudden diarrhea	Numerous colon and several Flexner colonies	Several large grayish colonies; many pin-point and many Flexner colonies
957	Sudden diarrhea	Numerous colon, a few proteus, and 2 or 3 Flexner colonies	Many colon, proteus, and several Flexner colonies
426	Bloody diarrhea	Numerous colon and a few Duval-Sonne colonies	Many pin-point colonies; dysentery colonies not found
287	Sudden diarrhea	Several colon, many pyocyaneus, and some other unidentified colonies	Very heavy growth of pyocyaneus colonies
180	Sudden diarrhea	Numerous colon colonies; nonlactose fermenting colonies not found	Several Flexner colonies and small colonies of lactose fermenters
512	Severe diarrhea	Numerous colon and a few proteus colonies; Flexner colonies not found	About 2 dozen Flexner and many unidentified colonies
820	Sudden nausea and diarrhea	Several alkalescens dysentery and many colon colonies	A few colonies of aerogenes and pin-point colonies
146	Sudden severe diarrhea	Many colon and several Flexner colonies	Many Flexner and some unidentified colonies
856	No data	Numerous colon but Flexner colonies not found	Two or 3 Flexner colonies and several colonies of aerogenes

yellowish colonies surrounded by a clear zone, while lactose fermenters form red or pink colonies surrounded by a cloudy zone.

From the results as shown in Table II, it is evident, where Flexner bacilli were recovered, that in nearly every instance growth was more abundant on desoxycholate-citrate than on MacConkey's agar. Flexner bacilli would not have been found in Nos. 117, 180, 512, and 856 if desoxycholate-citrate agar had not been employed. Specimen No. 820 is interesting since it apparently involved a rather severe case of *B. alkalescens* food poisoning.

During the past several months 59 stools have been received from 49 individuals, suffering from bacillary dysentery or diarrhea of sudden onset, and considered possibly suitable for cultural study. Dysentery bacilli were obtained from 39 specimens. One alkalescens and two Duval-Sonne cultures are included. *Endameba histolytica* was found in only one of these specimens.

TABLE III

COMPARATIVE FINDINGS WITH REGARD TO FLEXNER CULTURES

NO. OF EXAMINATIONS	CULTURES FROM MAC CONKEY'S AGAR	CULTURES FROM DESOXY-CHOLATE-CITRATE AGAR
56	20 or 36%	35 or 62%

It is interesting to note that in only one instance were Flexner bacilli found on MacConkey's when the corresponding desoxycholate-citrate plates gave a negative result.

Colonies superficially resembling those of dysentery bacilli, but which obviously lacked other cultural characteristics of the group, frequently were found in large numbers on desoxycholate-citrate plates. This disadvantage can be largely surmounted, we believe, by insisting on avoidance of gross contamination in collection of specimens.

Organisms having cultural and fermentative reactions characteristic of Flexner dysentery bacilli, but lacking in agglutinability, were rarely found. Antisera prepared against two locally obtained and carefully identified Flexner cultures generally sufficed for identification of new cultures. Frequently these two antisera gave only a slight cross agglutination.

DISCUSSION

During the course of this investigation Flexner bacilli were most frequently encountered and for their isolation desoxycholate-citrate agar definitely was superior to MacConkey's medium. This superiority seemed to depend upon inhibition of colon bacilli by desoxycholate-citrate medium, consequently permitting a relatively heavier seeding than was possible on MacConkey's medium. We have given considerable attention to descriptions of colonies, particularly those which are most often to be considered in inspecting desoxycholate-citrate plates, since unfamiliarity with types of growth and colonial characteristics is likely to be an important source of error and discouragement.

Since neither of two recently isolated Duval-Sonne cultures grew appreciably on desoxycholate-citrate medium, it will require further study to determine whether this medium is most suitable for cultivation of Duval-Sonne bacilli. The suppression of alkalescens bacilli on desoxycholate-citrate medium may not be advantageous if one departs from the usual viewpoint and considers these organisms of frequent significance.

All strains of dysentery bacilli, including stock cultures, which we have tested, have grown well on MacConkey's medium. We have found that it was wise to do confirmatory tests with colonies obtained from MacConkey's plates, or to use the utmost care in picking colonies from desoxycholate-citrate plates from which mixed cultures are all too easily obtained.

We have not encountered non-mannite-fermenting species, such as Shiga and Schmitz bacilli. None of our unidentified cultures were Newcastle bacilli.

CONCLUSIONS

The clinical consideration of acute diarrheal disorder should include bacteriologic investigation. In this study Flexner dysentery bacilli were cultured from a high percentage of stools obtained from patients suffering from acute diarrheal disorders. Desoxycholate-citrate medium was superior to MacConkey's agar for the isolation of Flexner dysentery bacilli.

Desoxycholate-citrate agar should be used in conjunction with a less restrictive medium in the quest for dysentery bacilli. Where typhoid and paratyphoid bacilli are to be sought, other and more suitable media for this purpose should be used.

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BLOOD TYPING AND CROSS MATCHING WITH PLASMA AND OXALATED ERYTHROCYTES*

TITUS B. RAY, MUNCIE, IND.

DURING the past year I have typed and cross-matched 750 blood specimens with oxalated erythrocytes and plasma along with the routine method employing serum and cells. The blood was obtained by venipuncture and oxalated. Two or three drops were pipetted into a tube containing approximately 1 c.c. of 3.5 per cent sodium citrate. The tube containing the oxalated blood was centrifuged for four minutes. The plasma was in one tube, and oxalated erythrocytes were suspended in 3.5 per cent sodium citrate in another tube. The procedure from then on was carried out as when using serum and cells.

Advantages.—1. With 750 blood samples I found a more rapid reaction present than when serum and citrated blood were used.

2. In doing routine work (such as complete blood counts, blood sugars, etc.) blood typing with oxalated blood and serum is very convenient.

*From the Ball Memorial Hospital, Muncie.
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3. In typing I have been using known types 2 and 3 plasma. I find that the plasmas have higher titers than the known types 2 and 3 sera.

SUMMARY

1. Seven hundred fifty specimens of blood were grouped and cross-matched using plasmas and oxalated erythrocytes suspended in 3.5 per cent sodium citrate.

2. These blood groupings and cross-matchings were checked with the usual routine method using blood serum and cells suspended in 3.5 per cent sodium citrate.

3. Identical results were obtained with each method, with a more rapid reaction using the plasma and oxalated erythrocytes.

A RAPID TECHNIQUE FOR SYPHILIS TESTING WITH FINGER BLOOD*

PAUL L. KIRK, PH.D., AND CLIFTON BENNETT, B.A., BERKELEY, CALIF.

THE simple and comparatively rapid modification of the Kahn test for syphilis, which was developed by Laughlen,[1] is increasingly becoming recognized as a dependable technique by clinical laboratories. Robinson and Stroud[2] confirmed its accuracy and there can be no doubt of its relative ease and speed as compared with other microtests, such as that of Kline,[3] and Eagle and Brand.[4] Detailed techniques for easy and rapid testing with finger and ear blood have not been described except in a few instances.[4, 5] These have not been as simple or as fast as the method described here, which was adapted from that previously developed[6] for use with quantitative drop analysis of finger blood. Since the Laughlen test seemed ideally adapted to use with small blood samples, it was used as the basic testing method. The complete method here described yields final results within a maximum of twenty minutes from the time of receiving a patient, and this time may often be appreciably shortened without sacrificing any step of the test. The advantage of this rapidity in case of emergency blood transfusions, as well as the general desirability of using capillary blood, e.g., with infants, is obvious. The simplicity of the technique makes accurate results available even to the relatively inexperienced operator.

EXPERIMENTAL

Two to 4 small drops of blood (approximately 0.1 c.c. or less), obtained by puncture of the finger or ear, were collected in a microcentrifuge cone of standard shape, 25 mm. in length and 6 mm. in external diameter, the total capacity

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of which was not more than 0.2 ml. The cone was dropped into the cup of an air-driven microcentrifuge previously described,[6] capable of running at about 25,000 r.p.m.* The tube did not require balancing, but was counterweighted by a similar tube in the opposite cup. The machine was run for three to four minutes, giving in that time ample amounts of clear serum.

The serum could be handled exactly as in the Laughlen procedure in which 1 loopful of serum is added to 2 drops of antigen. In this study a slight modification was introduced by the use of capillary pipettes of approximately known volumes for all measurements. That used for measuring antigen delivered about twice the volume of that used for serum, i.e., about 0.04 ml. of antigen and 0.02 ml. of serum. The antigen was first measured roughly into the depressions of a spherically ground slide, or better, two of the depressions of a porcelain spot plate. The serum was added to one of the drops of antigen, and a known negative control serum added to the other in all cases. The slide or spot plate was rotated in the hand to mix the serum and antigen thoroughly, and the time between additions of serum and appearance of visible clumping was noted. Usually visual observation was sufficient. In doubtful cases examination with a strong lens or the low power of the microscope was helpful. No actual disagreements were ever found between the two methods of observation. If no coagulation appeared in ten minutes, the test was negative. Strong positive reactions appeared in some cases within one minute. Tests appearing within five minutes were considered ++++, or very strong.

One hundred and twenty-five positive bloods obtained from the California State Public Health laboratory and the Clinic of the University of California Hospital were tested along with an equal number of known negative controls from various sources. In addition to known negative controls a considerable number of test blood samples found negative by the Kahn test were run along with their corresponding controls. The correlation with the results of the Kahn test run in the laboratories mentioned was exact in all but three cases, in all of which faint positives were obtained by this technique and negatives given by the Kahn test. This compares almost exactly with the results reported by Laughlen[1] and by Robinson and Stroud,[2] though the number of cases was too small to expect an exact statistical correlation.

Some results were obtained from saline extracts of dried blood and these were also exact as checked with hospital data.

The evidence here reported indicates that a dependable and simple test for syphilis may be easily run in a total of twenty minutes or less, with finger or ear blood, using the Laughlen antigen and a small high speed centrifuge.

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*Obtainable from Microchemical Specialties Co., 2112 Berkeley Way, Berkeley, Calif.

Acknowledgments are due the Lederle Antitoxin Laboratories for a supply of absolutely dependable Laughlen antigen; to the University of California Hospital and the California State Public Health Laboratory for syphilitic blood samples; and to Dr. Clarence Larson, formerly of the Mount Zion Hospital of San Francisco, for originally suggesting this development.

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THE DIAGNOSIS OF TUBERCULOSIS BY CULTURE AND GUINEA PIG INOCULATION*

Certain Methods Employed at the Los Angeles County General Hospital

Anson Hoyt, M.D., Frances Holtzwart, A.B., Barbara Kuntzner, A.B., and Roy T. Fisk, Ph.D., Los Angeles, Calif.

INOCULATION of guinea pigs for the diagnosis of tuberculosis has long been regarded as a method of great value and sensitivity. Cultural diagnostic methods have, however, only come into prominence within recent years. This report involves mainly the comparative results of diagnostic cultures and guinea pig inoculations for the identification of *Mycobacterium tuberculosis* that were run in parallel at the Los Angeles County General Hospital over a period of seven years (October 21, 1931, to October 21, 1938). Many types of specimens were submitted for diagnosis. Among these were the following:

(a) Sputum. These specimens were generally supposed to come from patients whose sputum had been negative for acid-fast rods on six smears.

(b) Urine. This was sometimes bladder specimens and sometimes catheterized separate samples from the right and left ureters.

(c) Feces, pus from draining abscesses or sinuses, biopsy or autopsy tissue specimens. All the above types of material were generally regarded as contaminated with organisms other than *M. tuberculosis* and were, therefore, treated to get rid of these contaminants before culturing and often also before animal inoculation.

(d) Specimens potentially sterile save for possible presence of *M. tuberculosis*. In this class were included spinal fluid, pleural fluid, peritoneal fluid, and fluid or pus aspirated from joints or other closed cavities. These specimens were generally not treated for contaminants but were inoculated as received into cultures and guinea pigs after a preliminary Gram stain had failed to disclose contaminating organisms.

*From the Laboratories of the Los Angeles County General Hospital and the Department of Bacteriology, School of Medicine, University of Southern California, Los Angeles.

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Herrold's medium was mainly employed for the cultures. This egg yolk agar medium was made up at first according to Herrold's original description.[1] Later the yolks of two eggs, instead of one egg yolk, were added to 150 c.c. of agar. The technique of making cultures, although modified slightly from time to time, was in effect as follows:

Material to be cultured was mixed with from one to two volumes of 5 per cent oxalic acid. This mixture was shaken vigorously by hand and incubated at 37° C. for a period ranging from forty-five minutes to two hours during which interval it was shaken several times. After incubation the mixture was neutralized with sterile 10 per cent sodium hydroxide to which bromthymol blue had been added as an indicator. The alkali was added until the indicator just remained slightly blue (approximately pH 7.0). When the volume of the specimens permitted, this acid treatment and neutralization were performed in sterile 50 c.c. centrifuge tubes. With larger specimens this preliminary treatment was performed in sterile flasks and then poured into centrifuge tubes. After neutralization the material was centrifuged at approximately 2200 r.p.m. for a minimum of thirty minutes. The supernatant liquid was discarded, and the sediment was used for making cultures and guinea pig inoculations. The cultures were run in triplicate and approximately 0.2 c.c. of the sediment was inoculated into each tube. The culture tubes were then placed on a rack in the incubator so that the slanted surfaces of the culture medium were level and the inoculated material maintained its distribution over the surface of each slant. At this time of primary incubation the culture tubes were plugged with cotton or, in later instances, with sterile unparaffined corks. After about four days, however, when the inoculum had dried sufficiently to remain fixed on the slants, lightly paraffined sterile corks were substituted for the cotton plugs (or unparaffined corks) of the culture tubes in order to minimize further drying, and the three tubes of each culture were fastened together with a rubber band and stored upright in the incubator. The cultures were inspected grossly twice a week, and characteristic colonies were stained by the Ziehl-Neelsen method. Treatment with acid and subsequent neutralization were often omitted in the case of uncontaminated material.

Guinea pigs were inoculated either intraperitoneally or subcutaneously in the groin, and the volume of the inoculum generally approximated 1 c.c. The latter method of injection appears preferable as with it one may often be able to demonstrate acid-fast rods in pus from a local lesion long before the general health of the pig begins to be affected. A small proportion of the pigs was tested with tuberculin before inoculation. The ideal procedure would be to make this a routine test. It was usually possible to keep the animals in individual cages, but at times the pigs had to be placed two in a cage. The pigs were generally examined for local lesions or swollen inguinal lymph glands at weekly intervals beginning at a period four weeks after inoculation. When pus could be expressed from a local lesion, this was smeared and examined for acid-fast rods. In all events the pigs were killed and autopsied at the end of eight or nine weeks after inoculation. A pig was declared positive for the presence of *M. tuberculosis* only when typical gross lesions were presented and when smears or stained sections from these lesions showed the presence of typical

acid-fast rods. Stained sections were made only when smears proved to be negative. Very occasionally it was impossible to demonstrate acid-fast rods either by direct smear or by section. In this case, if the lesions showed microscopic tissue changes suggestive of tuberculosis, a report of the probable presence of *M. tuberculosis* was turned in.

In the seven-year period covered by this report, 2,439 specimens were tested for the presence of *M. tuberculosis* by cultures and guinea pig inoculations run in parallel. With few exceptions, only one guinea pig was used on a specimen, whereas 3 culture tubes were inoculated. Out of these 2,439 tests, 345 were positive by animal inoculation, culture, or both methods. Table I shows the distribution of these positive results.

TABLE I

DISTRIBUTION OF 345 POSITIVE TESTS FOR THE PRESENCE OF M. TUBERCULOSIS

GUINEA PIGS POSITIVE	CULTURES POSITIVE	BOTH CULTURES AND GUINEA PIGS POSITIVE	GUINEA PIGS POSITIVE CULTURES NEGATIVE	GUINEA PIGS NEGATIVE CULTURES POSITIVE
301	242	198	103	44
87%	70%	57%	30%	13%

The results of these tests add evidence to the following suppositions which are upheld by many workers.

(a) Neither guinea pig inoculations nor cultural methods are infallible tests for the presence of *M. tuberculosis*. Both methods should be run in parallel whenever possible.

(b) Guinea pig inoculation appears to be a more sensitive method than culturing for the detection of *M. tuberculosis*.

In an attempt to determine upon a suitable culture medium, a series of parallel cultures of different specimens was run using both Herrold's egg agar medium and Corper's gentian violet potato medium.[2] By the time 24 specimens had grown up on Herrold's medium only 9 were positive on Corper's potato medium. Herrold's medium was then likewise compared with Corper's inspissated glycerol egg yolk medium.[3] Herrold's medium again came out ahead with 16 positive results against 8 for Corper's. After this comparison, Herrold's medium was used almost exclusively.

Herrold's medium can be very easily prepared. It has, however, certain theoretical disadvantages.

(a) It can be easily contaminated and contamination can sometimes occur by organisms originating within the egg inasmuch as this medium is not sterilized after the egg yolk is added. This objection has not proved serious in our experience, and very few batches of culture medium appear to have become contaminated in this manner.

(b) Egg yolk media dry out fairly easily. This tendency has been minimized by the use of paraffined corks as already described.

(c) Certain eggs may be infected with avian tubercle bacilli. If such eggs are used for making Herrold's medium, and the medium is then incubated, colonies of acid-fast rods may appear on it which are morphologically indis-

tinguishable from human or bovine strains of *M. tuberculosis*. In order to check on this possibility 7 of the positive cultures, in instances where the corresponding guinea pigs remained negative, were reinoculated into fresh guinea pigs, and all of these animals developed typical tuberculosis. We are aware, however, that various acid-fast saprophytes may at times grow up on cultures and confuse the observer. This is an occurrence which can be more easily controlled if cultures and pigs are run in parallel.

Positive results generally appear faster by culture than following guinea pig inoculation. Table II shows the distribution of times required for cultures and guinea pigs to become positive.

TABLE II

DISTRIBUTION OF TIME INTERVALS REQUIRED FOR CULTURES AND GUINEA PIGS TO SHOW THE PRESENCE OF M. TUBERCULOSIS

CULTURES	TIME INTERVALS	GUINEA PIGS
5 (2 per cent)	Less than 2 weeks	
7 (3 per cent)	2 weeks	
46 (19 per cent)	3 weeks	
75 (31 per cent)	4 weeks	7 (2 per cent)
47 (20 per cent)	5 weeks	9 (3 per cent)
42 (17 per cent)	6 weeks	37 (12 per cent)
13 (5 per cent)	7 weeks	62 (21 per cent)
5 (2 per cent)	8 weeks	158 (53 per cent)
	More than 8 weeks	28 (9 per cent)
2 (1 per cent)	Unrecorded times	
242	Totals	301

The use of cultures as a routine diagnostic method for tuberculosis requires considerable experience in order to obtain the best results. This is brought out in the series here reported when the 345 tests positive by culture, guinea pig inoculation, or both methods (Table I) are divided into (a) the first 150 positives and (b) the last 195 positives, upon which it appears that 93 out of the first 150 tests, or 62 per cent, gave positive cultural results, whereas 149 out of the last 195, or 76 per cent, showed growth of acid-fast rods on culture. The earlier records of our results fail to show the number of cultures that were reported as negative for *M. tuberculosis* because they had become contaminated by extraneous organisms introduced with the inoculum. In the last group of 1694 cases, however, 38 cultures (considering the 3 tubes as one culture) or 2 per cent of this series had to be discarded due to such contamination.

Guinea pig inoculation did not prove as uniformly efficacious a method for the identification of *M. tuberculosis* as we had previously considered it to be. In the first 150 of the 345 positive tests 139, or 93 per cent, of the pigs were positive, but only 162 out of the second 195, or 83 per cent, gave positive results. This drop in the efficiency of the guinea pig method as the series progressed was disappointing. It can probably be explained in part by the fact that an epidemic was killing a good many of the hospital's supply of pigs for a time and that, therefore, a number of pigs died too soon after inoculation to show definite positive or negative results. It was obvious, however, that every so often positive cultures would correspond with a pig that appeared

quite normal when autopsied eight weeks after inoculation; and, as stated previously, certain of these cultures on reinoculation into other guinea pigs produced typical tuberculosis. It is possible that these discrepancies may be accounted for by the distribution of organisms in the original suspected material. Our laboratory had no shaking machine to thoroughly homogenize the original specimens. A thorough shaking of these specimens with the acid used to eliminate contaminants should, in our opinion, eliminate a fair percentage of the discrepancies encountered between culture and guinea pig inoculation.

Murphy and Duerschner[4] (1938) reported a carefully performed comparison between the efficacies of cultural and guinea pig inoculation methods for the diagnosis of tuberculosis. They used slants of Petragnani's medium and inoculated them in triplicate with a total volume of material equal to that which was injected into the corresponding guinea pigs. Their results indicated that the cultural method possessed a sensitivity slightly greater than that shown by the method of guinea pig inoculation. One per cent of their complete culture sets had to be discarded due to contamination. They noted that the cultures became positive by microscopic examination about one week before macroscopic colonies appeared.

The results herewith reported do not uphold the contention that cultural methods are more sensitive than the inoculation of guinea pigs for the detection of *M. tuberculosis*. In evaluating our results, however, it must be remembered that the guinea pigs generally received larger volumes of inoculated material than that placed into the corresponding culture sets. When our positive tests are broken down into different groups of 50 consecutive cases, the following results obtain:

CASES		POSITIVE GUINEA PIGS	POSITIVE CULTURES
First	50	47	31
Second	50	46	31
Third	50	46	31
Fourth	50	38	40
Fifth	50	41	34
Sixth	50	47	41
Last	45	36	34

If the fourth 50 cases had been studied alone one would gain the impression that culturing was the most sensitive method, although the whole series gives evidence in favor of the guinea pig technique. It is possible that the series of Murphy and Duerschner might also have pointed in this direction had it been somewhat larger. The routine weekly microscopic examination of cultures before the appearance of gross colonies, as performed by Murphy and Duerschner, would appear to be an excellent technique to follow if time permitted.

One of us (Fisk) working in the laboratory of the contagious unit of the hospital, has developed a method of culturing spinal fluids which has proved useful in growing out many infectious agents including *M. tuberculosis*. The spinal fluid from a case of suspected meningitis is centrifuged at high speed for approximately thirty minutes. The supernatant liquid is discarded, and veal infusion broth containing 20 per cent filtered inactivated human serum is

poured over the sediment. The tube is then tightly corked and incubated. Among the pathogenic agents recoverable from spinal fluid by this method are meningococci, alpha and beta streptococci, staphylococci, pneumococci, *H. influenzae*, diphtheroids, *Coccidioides immitis*, and *Mycobacterium tuberculosis*. Most of these organisms appear rapidly. *M. tuberculosis*, however, takes two weeks or more to develop gross colonies. These colonies grow in the bottom of the culture and look like little flecks of wax. On smear they are composed of typical acid-fast rods. Several of these positive cultures have been inoculated into guinea pigs and have always brought about typical tuberculosis. All cultures positive for *M. tuberculosis* have corresponded to cases that were diagnosed as tuberculous meningitis either clinically or at autopsy. A large control series of spinal fluids taken from cases of various types of encephalitis, poliomyelitis, and various forms of neurosyphilis have failed to show growth of *M. tuberculosis* or other saprophytic acid-fast rods. This method for culturing many types of pathologic spinal fluids is reported here because it works very well in the case of tuberculous meningitis and because of its general applicability.

SUMMARY

Parallel cultures and guinea pig inoculations for the identification of *Mycobacterium tuberculosis* were run over a period of seven years. The animal inoculation method appeared to be the most sensitive. However, neither cultural nor animal inoculation methods were absolutely reliable, and it is suggested that both be performed as a check on one another whenever possible.

A simple method for culturing spinal fluid is described. This method favors the growth of many pathogens including *Mycobacterium tuberculosis*.

We gratefully acknowledge personal advice concerning the laboratory diagnosis of tuberculosis from Doctors H. J. Corper, Joseph E. Pottenger, C. Richard Smith, and Emil Bogen.

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DEMONSTRATION OF RABIES VIRUS IN GROSSLY DECOMPOSED ANIMAL BRAINS*

S. Edward Sulkin, Ph.D., and Nathan Nagle, A.B., St. Louis, Mo.

PUTREFIED brain tissue is usually difficult material in which to demonstrate Negri bodies[1] and for this reason the diagnosis of a decomposed brain by microscopic examination is unreliable. It is obvious, therefore, that a suitable and rapid animal inoculation method is desirable in order to obviate these difficulties. Only few attempts, however, have been made to sterilize decomposed animal brains without affecting the virus which is known to remain virulent in putrefied brain tissue for several days or even months.[2, 3] Mazzei,[4] for example, was able to demonstrate rabies virus in Berkefeld filtrates of tissue which had undergone putrefaction for sixty-three days. Other investigators[5, 6] have also successfully filtered rabies virus, but due to the inconsistency of the results this method is impractical in a health department laboratory.

In order to attempt isolation or demonstration of virus in decomposed brains it became necessary to remove the bacterial contaminants from the putrid material since intracerebral injection of such material into mice invariably produced death of the animal from intercurrent infection or from brain abscess.

In the preliminary experiments several bactericidal agents, including merthiolate, glycerin, phenol, and ether, were tried in an effort to find one which would sterilize the heavily infected inoculum without appreciably affecting the virus. Ether used according to the method suggested by Kramer and his associates,[7] Paul and Trask,[8] and others, proved to be the most desirable agent because its extreme volatality permitted prompt removal from the contaminated material after its bactericidal action had taken effect.

The following experiment was carried out to determine the bactericidal effect of ether upon contaminated dog brains. The horn (cornu ammonis) was removed from a grossly contaminated dog brain and ground in a mortar without an abrasive. Nutrient broth was added to make an approximate 20 per cent suspension which was then centrifuged at slow speed for about five minutes. The supernatant liquid was then divided into 7 aliquot portions, to 6 of which was added anesthetic ether to final concentration of 3, 6, 10, 12, 15, and 20 per cent, respectively. No ether was added to the first tube which served as control. The tubes were then thoroughly shaken, placed in the icebox (4° C.) and removed at intervals of one, two, six, eighteen hours to be cultured both aerobically and anaerobically on blood agar. The results of this experiment, sum-

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marized in Table I, and representing the average findings of several experiments, indicate that ether in final concentration of 10 per cent exerts a definite bactericidal effect upon contaminated dog brains after exposure for two hours at 4° C.

Having determined the optimum concentration of ether necessary to destroy contaminants in decomposed brains, it became necessary to establish whether this amount of ether would have any effect upon the virus.

TABLE I

BACTERICIDAL EFFECT OF ETHER UPON CONTAMINATED DOG BRAINS

	CONTROL NO ETHER		CONCENTRATION OF ETHER											
			3%		6%		10%		12%		15%		20%	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Exposure to ether at 4° C. for														
1 hour	+++	+++	+++	+++	+++	++	++	+	0	0	0	0	0	0
2 hours			++	++	++	++	0	+	0	0	0	0	0	0
6 hours			++	++	++	+	0	0	0	0	0	0	0	0
18 hours	+++	+++	++	+++	++	0	0	0	0	0	0	0	0	0

1 = 1 loopful plated on blood agar and cultured aerobically.
2 = Cultured anaerobically.
0 = No growth.
+ = 1-20 colonies.
++ = 20-100 colonies.
+++ = Heavy growth.

It is quite definitely established that ether has an attenuating effect upon the rabies virus only after long exposure.[9-12] Remlinger and his co-workers,[10] for example, studied the effect of ether on different strains of street and fixed virus and found that the brain tissue from the center of the hippocampus major (cornu ammonis) remained virulent after immersion in undiluted ether for one hundred twenty hours, while one strain of fixed virus remained virulent for one hundred sixty-eight hours.

The following experiment was designed to establish the concentration of ether that would have a definite effect upon the virulence of the virus after contact for eighteen hours. The horn (cornu ammonis) was removed from a dog brain suspected of being rabid. Two small pieces of horn were removed for microscopic examination (impression smears), and two additional pieces were preserved in full strength glycerin. The remaining portion of both horns was ground in a mortar without an abrasive, and sufficient nutrient broth was added to make an approximate 20 per cent suspension. After standing at room temperature for one hour, the suspension was centrifuged at slow speed in order to remove the large clumps. The supernatant liquid was then divided into 6 aliquot portions dispensed in sterile stoppered 15 c.c. centrifuge tubes. To 5 of these tubes was added anesthetic ether to final concentration of 7, 10, 15, 20, 30 per cent, respectively. The first tube received no ether and served as control. After thorough shaking, the tubes were placed in the icebox (4° C.) for eighteen hours, at which time they were centrifuged, and the supernatant liquid below the ether layer from each tube was injected intracerebrally into

each of 4 mice. In order to insure complete removal of the ether before the intracerebral injection was made, the tubes were placed in a desiccator for about five minutes.

TABLE II

EFFECT ON VIRUS AFTER EXPOSURE TO VARIOUS CONCENTRATIONS OF ETHER FOR 18 HOURS AT 4° C.

EXPOSURE TO DIFFERENT CONCENTRATIONS OF ETHER FOR 18 HR. AT 4° C.	DAY OF DEATH AFTER INTRACEREBRAL AND INTRAMUSCULAR INJECTION OF VIRUS	
	STREET VIRUS NO. 2715	STREET VIRUS NO. 2717
7 per cent	8+, 10+, 13,** 16+	4,** 9+, 11+, 16+
10 per cent	9+, 11+, 11+, 14**	8+, 11+, 13+, 13+
15 per cent	9+, 10+, 12+, 21+	9+, 9**, 12+, 14+
20 per cent	12+, 13+, 18+, 19+	1,* 15+, 15+, 18+
30 per cent	1,* 16+, 19+, 21-	17+, 21,** 26+, S
Control—No ether	8+, 10+, 10+, 15+	2,* 12+, 14+, 14+

*Animal died from trauma or of unknown complication other than rabies.
**Animal found dead and partially devoured. Examination could not be made.
+Negri bodies found on microscopic examination.
-No Negri bodies found on microscopic examination.
S = Animal survived (six weeks).

The results of this experiment, summarized in Table II, indicate that exposure to 10 per cent ether for eighteen hours at 4° C. had no effect upon the virulence of the two strains of street virus tested. Exposure to 30 per cent ether only slightly altered the virulence of these strains.

Having established that 10 per cent ether exerts a definite bactericidal effect upon contaminated dog brains after exposure for two hours at 4° C., and having determined that this concentration of ether does not effect the rabies virus under these conditions, a series of experiments was carried out using dog brains proving positive on microscopic examination. These brains were allowed to stand at room temperature until grossly contaminated. The experiments were conducted with the view of establishing a simple and practical method for treating contaminated brains before intracerebral injection into animals. The procedure used was as follows: The central portion of the hippocampus major (cornu ammonis) was dissected out, and a small piece was removed for microscopic examination and for glycerination. The remaining portion of the horn was emulsified in 10 c.c. of nutrient broth and allowed to stand for one hour at room temperature. The supernatant liquid (after larger clumps settled) was pipetted into a stoppered 15 c.c. centrifuge tube, and 10 per cent ether (about 1.0 c.c.) was added. The tube was then shaken thoroughly and allowed to stand in the refrigerator for two hours. After centrifugation at slow speed the supernatant liquid below the ether layer was removed for animal inoculation.

Nine grossly contaminated positive dog brains have been examined by the method described, and in each case a confirmatory diagnosis of rabies could be made by the mouse inoculation method.[13] The results summarized in Table III show that a positive report could be sent to the rabies clinic in an average of ten days after the animal was brought to the laboratory for examination. The method described has already been found practical in one specimen submitted for examination. A dog was brought to the laboratory on Aug. 15, 1938, and no satisfactory microscopic examination of the brain (No. 2741) could be made

because of gross decomposition. A positive report was sent to the rabies clinic on Aug. 26, 1938, eleven days after the ether-treated brain was injected intracerebrally in mice.

TABLE III

GROSSLY CONTAMINATED DOG BRAINS SHOWING NEGRI BODIES, EXPOSED TO 10 PER CENT ETHER FOR TWO HOURS AT 4° C.

DOG BRAIN NO.	DAY OF DEATH AFTER INTRACEREBRAL AND INTRAMUSCULAR INJECTION OF ETHER-TREATED DOG BRAINS			
2741†	11+,	13+,	13,**	15+
2743	9+,	10+,	12+,	12+
2747	1,*	4–,	12+,	S
2752	9+,	9+,	9+,	12+
2757	5–,	9+,	11+,	14+
2774	9+,	10+,	10+,	S
2793	10+,	10+,	12+,	12+
2796	9+,	10+,	10,**	11+
2800	11+,	13+,	13+,	14+
2803	1,*	10+,	11+,	12+

*Animal died from trauma or of unknown complication other than rabies.
**Animal found dead and partially devoured. Examination could not be made.
+Negri bodies found on microscopic examination.
–No Negri bodies found on microscopic examination.
†No satisfactory examination could be made of this brain because of gross decomposition.
S = Animal survived.

SUMMARY

Experiments are presented describing a simple procedure for demonstrating rabies virus in grossly decomposed animal brains. Of several bactericidal agents, including merthiolate, glycerin, phenol, and ether, the last mentioned proved to be the most desirable. It was found that ether in final concentration of 10 per cent exerts a definite bactericidal effect upon contaminated dog brains after exposure for two hours at 4° C. Exposure to this concentration of ether for eighteen hours at 4° C. had no effect upon the virulence of two strains of street virus tested. The experiments were conducted with the view of establishing a simple and practical method for treating contaminated brains before intracerebral injection into animals.

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CLINICAL DEMONSTRATION OF IRON IN THE SKIN IN HEMOCHROMATOSIS*

HAMILTON R. FISHBACK, SC.D., M.D., CHICAGO, ILL.

THE diagnosis of hemochromatosis is verified by the finding of iron deposited diffusely in the skin. The visual evidence of its presence, that is, the pigmentation, may vary considerably in intensity and in its shading from light yellow brown, deep bronze, or bluish brown, to slate color. The two conditions with pigmentation most likely to be confused with that of hemochromatosis are Addison's disease and argyria. While clinical study may serve in differentiation, yet a positive ruling on the presence of dermal iron is the simplest and most direct finding.

The histochemical demonstration of iron has been done by the excision of a piece of skin, fixing it, sectioning, and exposing the sections to the action of chemicals which develop the iron colorimetrically. While this is not a formidable procedure, it is something of a nuisance to both the patient and the doctor, and consequently is used very seldom. The time required for a report may vary, but under common working conditions it generally ranges from two to five days.

A man with hemochromatosis was seen recently with the familiar triad of findings: pigmentation of the skin with iron demonstrated histologically, diabetes mellitus, and enlarged cirrhotic liver. Some of the disadvantages of the old procedure for demonstrating iron in the skin were brought to attention and a new method was devised.

Equal parts of sterile solutions of 0.5 per cent potassium ferrocyanide and one-hundredth normal hydrochloric acid were mixed and injected intradermally so as to form a wheal.

A slight blue color was evident almost immediately, which darkened to a deep blue within five minutes. It is possible that the color became somewhat darker over the next few hours, but the change was insignificant. A narrow red zone appeared at the periphery of the wheal in about two days. This persisted throughout the slow contraction of the blue test spot until its disappearance in about two weeks.

*From the Department of Pathology, Northwestern University Medical School, Chicago.
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The negative test showed a white wheal which later developed the peripheral red zone and disappeared in about the same time as a positive test.

There is a sharp stinging sensation with the injection, but it ceases within a few seconds. There is no pain or itching afterward, as might be developed with acute inflammation. The skin remains intact without peeling or necrosis.

Hemorrhage into the tissues from bruises or other cause might result in iron deposit, but these causes are local and usually can be ruled out easily.

In post-mortem examination in the case here cited the iron test was positive in the skin of any chosen area of the body or extremities, even in parts where no altered pigmentation was visible. It is more difficult to inject the solution intradermally after death, although such localization is necessary for development of the color.

SUMMARY

A test for iron in the skin is described, making a simple application of the familiar potassium ferrocyanide chemical reaction for iron.

It is without any evident injurious local or general effects.

The test can be performed and read within a few minutes in the doctor's office.

Since diffuse iron deposit occurs only in hemochromatosis, this test would appear to be specific for that disease.

In hemochromatosis the test is also positive after death.

Grateful acknowledgment is made here to the Davella Mills Cancer Foundation Fund of Wesley Memorial Hospital of Chicago, which provided hospital care for the patient studied.

A RING TEST FOR URINE BROMIDES*

ALEX BLUMSTEIN, M.D., MINNEAPOLIS, MINN., AND PAUL M. ZOLL, M.D., AND JESSE J. MAYER, B.S., NEW YORK, N. Y.

IN MENTAL patients the question of bromism arises frequently.[1] Wuth[2] in 1927 described a test to detect the presence of bromides in the urine as follows:

To 25 c.c. of urine add 1 gm. of animal charcoal. Mix well. Allow to stand for a few minutes and filter. To exactly 5 c.c. of filtrate add 1 c.c. of 20 per cent trichloracetic acid and 1 c.c. of 0.5 per cent gold chloride. A brown shade indicates the presence of bromides. Iodides will be indicated by a precipitate. For comparison make the following solution: 0.5 c.c. of 0.1 per cent sodium bromide, 4.5 c.c. of water, 1 c.c. of 20 per cent trichloracetic acid and 1 c.c. of 0.5 per cent gold chloride.

*From the Division of Nervous and Mental Diseases, University of Minnesota Medical School, Minneapolis, and the Departments of Psychiatry and Pathology, Bellevue Hospital, New York.

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It seems to us that a simpler procedure than that recommended by Wuth is desirable for routine clinical usage. The following ring test has proved satisfactory in our hands:

Roughly 1 c.c. of 20 per cent trichloracetic acid is mixed with approximately 5 c.c. of urine, and the mixture is overlayered with 0.5 per cent gold chloride.

If bromide is present in concentrations over 50 mg. in 100 c.c. of urine, a yellowish to a reddish orange ring develops almost immediately at the interface. Occasionally the ring will have a brownish tinge, but that is not the predominant color. The characteristic color reaction is not obscured even in highly pigmented urines. The test is difficult to read in artificial light unless a daylight bulb is used. To become familiar with the color of the ring, one need only perform the test on urines containing various concentrations of bromides. At 50 mg. per cent a positive test first appears as a canary yellow ring; at 80 mg. per cent, the color obtained is orange; at 160 mg. per cent, the ring is reddish orange. With higher bromide concentrations the reddish orange ring becomes more intense. Not infrequently a grayish precipitate forms at the interface of the gold chloride and urine, and occasionally a purple color appears below the interface. These reactions should not be interpreted as positive tests in the absence of the characteristically colored ring. Bile and iodides, if present in the urine, interfere with the test. Urines containing a high concentration of bile and bromides give a negative test. Iodides in a urine containing bromides mask the color of the ring by a yellowish gray precipitate which is immediately formed. The iodide reaction is readily distinguishable from the bromide reaction, but it will mask bromides if present.

In order to determine the clinical limitations of the procedure, the ring test was done on urines of patients receiving therapeutic doses of sodium bromide. Five patients received 0.6 gm. of sodium bromide three times a day. Urine tests became positive on the fourth day of medication in 2 cases; on the fifth day in 2 cases, and on the seventh day in one case. Twenty patients received 1 gm. sodium bromide three times a day. The urine test was positive after one day of medication in 2 cases; two days in 6 cases; three days in 6 cases; four days in 4 cases; and five days in 2 cases. In no instance did the urine test remain negative following the usual therapeutic medication.

The test was done routinely on urines from patients on the medical wards of the Psychiatric Division of Bellevue Hospital. Nine hundred and sixty-six urine specimens were tested; 164 gave a positive ring test for bromides. In every positive test the patient had either been given bromides in the hospital, gave a definite history of bromide ingestion, or had over 75 mg. per cent bromides in the blood serum. Using those features as criteria, no false positives were discovered in the 966 urine specimens.

In 520 consecutive cases admitted chiefly to the medical wards of the Psychiatric Division of Bellevue Hospital, the ring test was positive for urinary bromides in 23 patients. In 13 cases bromide ingestion was not suspected before the urine bromide test was done. Simultaneous blood bromide determinations[3] and urinary tests were done on 97 patients. In every instance where the urine test was positive the serum bromide level was above 75 mg. per cent. Seven showed serum bromide levels above 75 mg. per cent. In only one case was

the ring test in the urine negative when the serum bromide level was above 75 mg. per cent. In this case the serum bromide on admission was 125 mg. per cent, at which time the ring test was positive. After 10 days the urinary test became negative, although the serum bromide level persisted at 100 mg. per cent. This patient was a diabetic who had bouts of vomiting and took salts and fluids poorly.

The ring test may also be done on spinal fluid and blood serum. One need only overlayer spinal fluid with 0.5 per cent gold chloride solution and look for the characteristically colored ring at the interface. For a qualitative blood bromide test the serum proteins are precipitated by 20 per cent trichloracetic acid and spun down by centrifuge. The supernatant fluid is overlayered with 0.5 per cent gold chloride. For example, in one case, the serum bromide was 200 mg. per cent; the ring tests on serum and spinal fluids were positive.

COMMENT

The test here described has been useful to us as a routine procedure to detect the presence of bromides in urine. When the urine test is positive, a quanitative blood bromide determination is done. In our experience a negative urine test is strong evidence against bromism. In cases of suspected bromide intoxication it offers a quick method of confirming the diagnosis. The test can easily be performed as part of the routine urine examination. The necessary solutions are stable and easily prepared.

CONCLUSION

A ring test for urinary bromides is described. It is suggested that the test can be used as a routine procedure on all patients entering mental hospitals, or whenever indicated in general hospitals or office practice.

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PREPARATIONS FROM THE SPINAL CORD IN THE LABORATORY DIAGNOSIS OF RABIES*

ROBERT A. GREENE, PH.D., AND EDWARD L. BREAZEALE, B.S.A., TUCSON, ARIZ.

IN THE laboratory diagnosis of rabies, preparations are usually made from the hippocampus major, the gray matter of the cerebellum or the cortex in the region of the fissure of Rolando, because Negri bodies usually occur most abundantly in these areas. Park, Williams, and Krumwiede[1] have stated that they "have always found bodies in the spinal cord in abundance, but here they are especially prone to be localized in discrete groups of cells."

To our knowledge preparations from the cord are not usually employed for the demonstration of Negri bodies. In most of the specimens received at the Arizona State Laboratory for the laboratory diagnosis of rabies, the head has already been removed, and the cord is readily accessible. It occurred to us that the use of the cord for this purpose would result in a considerable saving of time, and would be particularly valuable in cases where the animal had been shot through the head.

We have adopted the following procedure when the head has been removed: Preparations are made from the cord and are stained in the usual manner. If Negri bodies are demonstrated, no further examination is made. If Negri bodies are not found in the cord, then preparations are made from Ammon's horn. So far, the results of examinations of preparations from the cord and from Ammon's horn have agreed perfectly. We feel, however, that in the event that Negri bodies are not demonstrated in the cord, preparation should be made from the brain.

The use of the cord for this purpose results in a saving of time, particularly in the case of larger animals, where the removal of the brain is not always easy.

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*From the Arizona State Laboratory, Tucson.
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A METHOD OF COUNTING BLOOD PLATELETS

THOMAS F. WALKER, M.D., AND PATRICIA A. SWEENEY, B.A.,
GREAT FALLS, MONT.

THE numerous methods that have been proposed for counting blood platelets, and the fact that the normal count differs with the method used, seem to indicate that an entirely satisfactory method has not been devised.

Our experience indicates that the normal number of platelets varies not only with the method used, but also with the technician making the count. Where it is necessary to make a series of platelet counts upon a patient, we insist that all counts be made by the same technician.

In few, if any, laboratories is a platelet count among the procedures carried out as a routine in doing a complete count. Yet, we feel that the platelet count is of sufficient importance to warrant its inclusion in routine "complete blood counts," and that if this were done, our knowledge concerning the significance of variation in the number of platelets would be materially increased. Doubtless, if a simple, accurate method of counting platelets were available, it would be used as a routine.

We have found the method of making plasma platelet counts as devised by Nygaard the most satisfactory method in use at the present time. However, since it requires venipuncture, and since the syringe is filled with a more or less toxic solution, it is seldom used as a routine procedure. In addition, the time required for the sedimentation of the red blood cells is a disadvantage.

We have found that the majority of difficulties in making platelet counts are overcome by the use of the following method:

1. Before making puncture in finger or ear, draw a 1.1 per cent solution of sodium oxalate into white blood cell pipette to the mark 1 and expel it by blowing. This moistens the pipette and prevents platelets from adhering to the glass.

2. Quickly draw blood to the mark 0.5.

3. Quickly draw diluting fluid (1.1 per cent solution of sodium oxalate) to the mark 11, and mix by shaking vigorously.

4. Place a heavy rubber band around pipette so as to close ends.

5a. Place pipette in centrifuge and centrifugalize for the shortest possible time necessary to drive red blood cells into stem of pipette. (We have found that one-half minute at a speed of approximately 1,600 revolutions will accomplish this without changing the platelet count) or

5b. Let pipette stand upright for two hours to permit the red blood cells to settle into the stem of the pipette. (There is some variation in time depending on the caliber of the lumen in the stem of pipette.)

6. Gently expel the red blood cells from the stem of the pipette by blowing.

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7. Place a drop of clear supernatant solution in counting chamber (a few red blood cells will do no harm).

8. After ten minutes count all the platelets in 80 small squares (4 groups of 20 small squares). Because of the slowness with which platelets settle, it is very important to focus carefully over each square counted so as to count platelets at different levels in the chamber.

9. Add three zeros to the total count for the number of platelets per cubic millimeter of blood. (We have considered 300,000 as the normal number per cubic millimeter, using this method.)

In step 1, we at first filled the pipette with the diluting fluid to mark 1 and then drew the blood into the pipette to the mark 0.5, as directed in some of the older methods. However, we found that the blood and the solution tended to blend, making it difficult to draw in the correct amount of blood. When the pipette was simply moistened, the blood could be accurately drawn to the mark 0.5, and the variation between counts on the same patient became less, indicating greater accuracy.

Using this method, we feel that it is possible to count platelets with a percentage of error not much greater than in counting red blood cells. The amount of work is not appreciably greater than that involved in doing a red blood cell count.

ZONE REACTIONS IN THE KLINE TEST*

ROBERT A. GREENE, PH.D., AND EDWARD L. BREAZEALE, B.S.A., TUCSON, ARIZ.

IT IS frequently observed in serologic reactions that lower dilutions of serum may give negative results, while higher dilutions of the same serum may give strongly positive reactions. Zone reactions are frequently encountered in the Wassermann test (an excellent discussion is given by Eagle[1]), but to our knowledge have not been observed frequently in flocculation tests for syphilis, especially in those tests where a single fixed antigen-serum ratio, is employed.

At the Arizona State Laboratory the more common serologic tests for syphilis have given excellent agreement.[2] Occasionally, we have noted a serum which might give a doubtful or weakly positive Kline reaction and strongly positive Kahn, Hinton, or Wassermann reactions. At first these were considered as technical errors, but when several of these Kline-doubtful-Kahn-positive sera were examined in two different laboratories, by three different persons who employed different lots of LaMotte Kline antigen, we concluded that these results were not necessarily technical errors.

It appeared to us that these discrepant results might be due to a zone effect which was not appearing in the Kahn test because, in the latter, three tubes of varying antigen-serum ratio are employed. In order to determine whether these results might be due to a zone effect, these sera were diluted with physiologic saline, and the Kline test was performed upon the diluted sera. At the

*From the Arizona State Laboratory, Tucson.
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same time a large number of negative sera were diluted and examined in the same manner as the positive sera. In every case these negative sera gave uniformly negative Kline reactions.

Table I gives some typical results of the examination of these sera.

TABLE I

KLINE REACTIONS ON DILUTED SERUM

SERUM NO.	KAHN (UNDILUTED SERUM)	KLINE (UNDILUTED SERUM)	KLINE					
			1:1	1:2	1:4	1:8	1:16	1:32
110	3 plus	1 plus	2 plus	4 plus	4 plus	2 plus	1 plus	Doubtful
630	2 plus	1 plus	1 plus	3 plus	4 plus	3 plus	2 plus	Doubtful
1508	4 plus	1 plus	2 plus	3 plus	1 plus	Doubtful	Negative	Negative
1576	4 plus	Doubtful	3 plus	1 plus	Doubtful	Negative	Negative	Negative
1681	4 plus	1 plus	3 plus	1 plus	Doubtful	Negative	Negative	Negative
1828	4 plus	Doubtful	2 plus	2 plus	3 plus	Doubtful	Negative	Negative
1855	4 plus	1 plus	1 plus	2 plus	1 plus	Doubtful	Negative	Negative
9805	4 plus	2 plus	3 plus	4 plus	3 plus	1 plus	Negative	Negative
9937	4 plus	2 plus	2 plus	3 plus	3 plus	2 plus	1 plus	Doubtful

An examination of Table I reveals that when the sera were diluted, stronger reactions were observed in the higher dilutions than in the undiluted sera. In general, the Kline results on serum dilutions of 1:1 or 1:2 agreed well with the Kahn results on the undiluted sera. Negative sera, treated in the same manner, gave uniformly negative results. Several hundred other sera, which gave comparable Kline, Kahn, Hinton, and Wassermann reactions, have been examined in the same manner, but zone effects have not been observed.

Although the Hinton test is not read in terms of degrees of positivity, we have, in a few cases, noted zone effects in diluted sera. That is, a more abundant floc is formed in the diluted sera than in the undiluted.

The incidence of the sera which show zone effects is (in our experience) not great—approximately 1 per 1,000—and should not prove a great source of error, unless only one test is employed in the laboratory diagnosis of syphilis. These results emphasize the desirability of employing more than one technique, and particularly the desirability of employing either dilutions of serum or one test in which the antigen-serum ratios are varied.

CONCLUSION

It was found that sera which gave strongly positive Kahn and weakly positive Kline reactions showed a zone effect when the serum was diluted with saline. In general, such sera, when diluted 1:1 or 1:2, gave Kline reactions which compared to the Kahn reactions with undiluted sera.

The incidence of these sera is not great (approximately 1 per 1,000).

REFERENCES

1. Eagle, H.: The Laboratory Diagnosis of Syphilis, St. Louis, 1937, C. V. Mosby Co.
2. Breazeale, E. L., Greene, R. A., and Harding, H. B.: A Comparison of the Eagle, Ide, Kahn, Kline and Laughlen Tests for Syphilis, Southwest Med. 22: 311, 1938.

Since this manuscript was submitted, our attention has been called to similar observations by Dr. A. S. Wiener, J. LAB. & CLIN. MED. 22: 1069, 1937.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

EMBEDDING OVEN, an Inexpensive, Bouton, S. M., Jr. Arch. Path. 27: 58, 1939.

The oven consists primarily of a box constructed throughout of 1⅛ inch (2.8 cm.) lumber, thus utilizing the insulating qualities of wood. The door occupies the entire frontage, is completely recessed into the frame of the box, and consists of a wood frame holding two panes of glass, separated from each other by an intervening air space. The door is supplied with an inexpensive catch lock. The inside of the box is equipped a short distance from the bottom with four regulation screw sockets, two in each side wall, to hold four electric light bulbs (in this laboratory, two 25 watt and two 50 watt bulbs); and the outside, with individual switches, one for each bulb. The electric cord leaves the box at the rear. The top of the box is perforated by six ½ inch (1.3 cm.) holes (the number and diameter of these holes may, of course, vary) within a rectangular space large enough to accommodate a dehydrating jar. This space is marked off by a low wooden frame to hold the jar; the frame, open at the rear, furnishes guides for a sliding panel which can be adjusted to cover from one to all six holes. For this purpose, the latter are staggered.

The inside of the box is further equipped with rails along both sides, not too close to the bulbs, to hold two removable stout wire mesh trays. A centigrade thermometer is hung on the inside of the door, its mercury bulb insulated against the glass by a piece of very thick cardboard. Thus the temperature within the oven can be read at a glance without opening the door. For purposes of comparison and checking, another thermometer may be attached to the rear wall in such a manner that it also may be read through the closed door.

CLOT RETRACTION, A Simple Method for Measuring, MacFarland, R. G. Brit. Lancet 1: 1199, 1939.

The apparatus required for measuring clot retraction consists only of a graduated centrifuge tube with the scale marked in 0.1 cm. divisions, a glass rod, and a cork to fit the mouth of the tube, bored to receive the rod as an easy sliding fit. The rod is about ½ inch longer than the tube and has a small "button" expansion formed about ½ inch from one end.

The retraction is measured as follows: More than 5 cm. of blood is obtained by venepuncture. The centrifuge tube is filled from the syringe up to the 5 cm. mark. The glass rod is put into the tube, so that the expansion is immersed in the blood, and the cork is fitted, the projecting end of the rod passing through the hole bored to receive it. The tube is then placed in a water bath at 37° C. and examined from time to time for coagulation of the contained blood. An hour after firm clotting it is removed from the bath. If retraction has taken place, the clot will be seen to have shrunk away from the walls of the tube and to be attached to the rod only. By sliding the rod upwards through the cork, the clot, supported on the expansion, can be raised clear of the expressed fluid, so that the volume of the latter may be measured directly on the graduated scale.

Retraction is expressed in terms of this volume, as a percentage of the original volume of blood. Thus, if 5 cm. of blood after clotting express 3 cm. of fluid, the retraction is

$$\frac{3 \times 100}{5} = 60 \text{ per cent.}$$

In measuring retraction in this way it is necessary to ensure that the graduated tubes are perfectly clean and are unscratched inside; otherwise the clot may stick to the glass and

prevent retraction. Clots that have stuck may be freed by carefully passing a fine stiff wire round the inside of the tube, keeping it in contact with the glass. The tube should then be incubated for a further period of an hour.

From a study of 50 normal donors (23 female, 27 male) the normal range by this method was 43.9 to 66.5 per cent.

In a series of 250 pathologic cases it was found that retraction might be reduced in jaundice, lobar pneumonia, myelomatosis, and other conditions, apart from those with an obvious reduction in the platelets.

In the hemorrhagic states, retraction was normal in hemophilia, telangiectasis, and athrombopenic purpura hemorrhagica. Only in thrombopenic purpura was it reduced.

SULFANILAMIDE. A Study of Its Mode of Action on Hemolytic Streptococci, Keefer, C. S., and Rantz, L. A. Arch. Int. Med. 63: 957, 1939.

From a study of the mode of action of sulfanilamide in vitro and in vivo, the following facts emerged:

When sulfanilamide was added to whole defibrinated blood so that the concentration was 7 mg. or more per 100 c.c., there was definite bacteriostasis, and in some instances a definite bactericidal effect was demonstrated.

We were unable to demonstrate a bactericidal effect of sulfanilamide when it was added to plasma, although bacteriostasis was present in plasma containing sulfanilamide. This bacteriostasis was less striking in plasma than it was in whole blood.

The samples of blood which showed a bactericidal effect with sulfanilamide were those containing some natural antibodies. It is suggested, therefore, that sulfanilamide may enhance the bactericidal effect of whole blood provided natural antibodies are present.

In the cases in which it was not possible to demonstrate a bactericidal effect, a bacteriostatic effect was shown. When the number of organisms was small, there was almost complete bacteriostasis; when the inoculum was larger, the rate of multiplication was slower and the number of organisms at the end of twenty-four hours was less than in the controls.

It appears that antibodies are important in destroying hemolytic streptococci in vitro even in the presence of sulfanilamide.

The principal action of sulfanilamide in vitro is to slow the growth of the organism. It has no direct bactericidal effect on any serologic type of hemolytic streptococci.

LEUKOCYTOSIS, Neutrophilic, in Spinal Fluid Associated With Cerebral Vascular Accidents, Townsend, S. R., Craig, R. L., and Braunstein, A. L. Arch. Int. Med. 63: 848, 1939.

Six cases of cerebral softening and cerebral hemorrhage in which a great excess of polymorphonuclear leucocytes was found in the spinal fluid are described.

The authors believe that this leucocytosis is due to an aseptic meningeal reaction to adjacent areas of necrosis within the brain.

It is only in cases in which the lesion is close to the surface of the cortex or approaches the wall of the ventricle that leucocytosis occurs, for deeply seated lesions cause no such reactions in the spinal fluid.

The leucocytosis usually begins within several hours after the infarction and gradually disappears within the next five to six days. The number of cells varies between 50 and 3,000 or more per cubic millimeter.

In cases in which the leucocytosis is great there may be stiffness of the neck and a positive Kernig sign, i.e., clinical evidence of meningeal irritation.

In cases in which the vascular lesion does not cause demonstrable focal signs leucocytosis of this type in the spinal fluid may result in an erroneous diagnosis of meningitis.

Other writers have made similar observations, and a brief discussion of the literature is appended.

PHOSPHATASE, Serum, Influence of Liver on, Schiffman, A., and Winkelman, L. Arch. Int. Med. **63**: 919, 1939.

Serum phosphatase values increase more than tenfold after ligation of a single hepatic duct. No other important changes in the blood result from this procedure.

After the rise that follows obstruction of a single hepatic duct, the values steadily decrease and in thirty to fifty-five days return to normal limits.

Obstruction of the common bile duct in a dog causes a rise in serum phosphatase more than twice that which follows a similar obstruction in other dogs whose livers have been damaged by intravenous administration of arsenic trioxide.

The resultant values of serum phosphatase in hepatic and biliary disorders are dependent on two equally important factors: (a) the degree and extent of biliary obstruction and (b) the functional state of the liver cell.

TRICHINOSIS, Manifestations of, in the Central Nervous System, Evers, L. B. Arch. Int. Med. **63**: 949, 1939.

A case of trichiniasis which presented a clinical picture of toxic encephalitis and neuroretinitis, with subsequent recovery, is reported. Motile larvae were isolated from the spinal fluid after the encephalitis signs had largely disappeared.

Review of the literature reveals that the presence of larvae in the spinal fluid does not always produce clinical manifestations of involvement of the nervous system. Larvae of *T. spiralis* in the spinal fluid have been reported in 24 patients. Of these, 4 were fatal, a mortality rate of 26.6 per cent.

From the reported cases it seems probable that careful examination of the spinal fluid in suspected cases of trichiniasis, with or without symptoms of involvement of the central nervous system, may aid in the diagnosis of this disease.

HYPERTHERMIA, Therapeutic, Pathological Changes Following, Lichtenstein, L. Am. J. Path. **15**: 363, 1939.

A description is given of the changes observed at autopsy in a case of uncontrollable hyperpyrexia (109° F.) ensuing upon hyperthermia treatment for arthritis of the finger joints. The hyperpyrexia (which developed in the course of the third of a series of treatments) was associated with coma and respiratory failure, and the patient died about thirty-five hours after the fever was initiated. In this case the significant pathologic changes were the following: (1) multiple punctate hemorrhages and necrobiosis in the gray matter of the cerebral cortex; (2) hemorrhage in the left internal capsule; (3) thrombosis of venules and capillaries in the cerebral cortex and internal capsule; (4) cerebral congestion and edema; (5) infarction of kidneys and spleen; (6) marked hepatic degeneration and edema; and (7) pulmonary congestion, hemorrhage, and edema.

The changes found in this case have been correlated with, and discussed in relation to, those in 9 cases previously recorded in the literature. Certain of the changes seen in the case reported here, notably the vascular lesions, have not hitherto been described in connection with fatalities following fever therapy. Specifically there seems to be no previous description of the thrombosis of venules and capillaries in affected portions of the brain, and of the infarcts in kidneys and spleen, apparently due to focal necroses of small arterial branches of these organs.

The principal complications and sequelae of hyperthermia, and especially its effects upon the brain, blood vessels and liver, are indicated. Attention is also drawn to the fact that the reactions to therapeutic hyperthermia are sometimes serious even when they are not fatal.

LEAD, in Human Tissues, Bagchi, K. N., Ganguly, H. D., and Sirdar, J. N. Indian J. M. Research **26**: 935, 1939.

Lead is normally present in almost all human tissues in variable quantities. Individual variation in the amount of lead is due to difference in the quality and quantity of food ingested. The lead content of normal tissues of the Europeans, as observed by

the European workers, is much higher than that of Indians. The nature of diet and the conditions of living in the West are responsible for this difference.

The dithizone method has been employed for determination of lead in this investigation. It has been proved by the American workers that this method is as good as the spectrographic method, provided the amount of lead to be estimated exceeds 0.001 mg.

Bone, tooth, and hair retain large quantities of lead. The maximum amount is found in hair, especially on the black hair of Indian women. The color of hair appears to depend on its lead content. The skin, in spite of the fact that it is histologically closely related to hair and contains numerous hairs and hair follicles, is very poor in lead.

The ovary is the only organ which has been found free from lead, and thus it differs from testis which contains quite an appreciable amount. The foetal tissues do not show any affinity for lead, although it is believed otherwise.

In cases of abnormal exposure to lead, the liver, stomach, kidney, and lungs show well-marked increase in their lead content, and thus indicate the route through which lead gets into the system.

CHOLERA, Differential Isolation of Vibrio Cholerae, Read, W. D. B. Indian J. M. Research **26**: 851, 1939.

Various methods are described and investigated with a view to the differential isolation of *V. cholerae* from mixtures of cultures, stools, and natural and artificial waters inoculated with *V. cholerae*.

A modification of the bismuth sulfite enrichment medium of Wilson and Blair was the best and enabled the vibrio to be isolated from an inoculum that would just grow only in ordinary broth.

By the use of this medium mannose-fermenting vibrios can be successfully differentiated from nonmannose-fermenting vibrios and from coliform types. Other common water and stool organisms except streptococci are suppressed, but no method of facilitating *V. cholerae* as against mannose-fermenting inagglutinable vibrios was discovered. The value of the method will depend on whether the mannose-fermenting vibrios found in natural sources can outgrow *V. cholerae* or not. The difficulty due to the growth of total organisms when ordinary peptone water enrichment is employed is overcome.

CALCIUM, Serum, in Newborn, Denzer, B. S., Reiner, M., and Wiener, S. B. Am. J. Dis. Child. **57**: 809, 1939.

A study of the calcium level in the cord blood and of the blood calcium curve of the neonatal period has been made.

There is a distinct drop in the calcium level of the blood during the first four days of life, with a subsequent slow rise to a point slightly above the average level of later infancy. This depression of the calcium content is not related to race, weight at birth, or neonatal loss of weight.

The inorganic phosphorus content of the blood during the neonatal period is higher than the phosphorus content of the cord blood but shows no definite constant curve.

The protein level of the blood remains constant during the neonatal period and, therefore, cannot affect the level of the available ionizable calcium.

The data derived from a comparison of calcium and phosphorus curves in the neonatal period do not supply an adequate explanation of tetany neonatorum.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Anemia in Practice*

THE work of Minot and Murphy in the development of liver therapy in anemia is now a part of medical history and more than suffices to establish the competence of the author to discuss anemia and its treatment.

The outstanding feature of this book is its not only clear and succinct style but also its eminently practical character. While of definite interest and value to the clinical pathologist and hematologist, it may be regarded as a "must" item for the physician's library as a text abounding in clinical implications and applications. There can be no clinical confusion about anemias and their management for the reader of this book.

There are two main sections covering Hypochromic and Normocytic Anemias and Pernicious Anemia. Both are excellent, clear, tersely expressed and yet comprehensive.

This book can be highly recommended.

Experimentelle und klinische Ergebnisse mit der Friedmannschen Tuberkulosevaccine†

THE author reports in a book of 155 pages his experiences with Friedmann's vaccine in animal experiments and in clinical observations in human cases of pulmonary tuberculosis. The results are completely negative, and he shows repeatedly the inefficiency of Friedmann's treatment by detailed accounts of each animal experiment and each human case. He reports on 6 rabbits, 50 guinea pigs, and 126 cases in man. He finds that Friedmann's turtle vaccine does not produce an active immunization against tuberculosis in warm-blooded animals. The purpose of the book is to counteract the unjustified recommendation of Friedmann and his followers.

The book does not add anything new to the extensive experience of other investigators on this same subject, and it might well have been much shorter.—*Lotte Kimmelstiel.*

Vitamins and Vitamin Deficiencies‡

THOUGH the vitamins are a relatively recent discovery, the literature has reached such astonishing proportions that one who seeks information may find it almost overwhelming.

In this volume, apparently the first of a series, Dr. Harris has presented in a crisp, succinct, and exceedingly interesting manner a historical and introductory account of vitamins B and B_1.

Dr. Harris is not only admittedly competent to discuss this complex subject with authority but, in addition to a thorough familiarity with the literature, has a distinct flair for developing the interest and even drama of his subject.

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*Anemia in Practice. Pernicious Anemia. By William P. Murphy, A.B., M.D., Associate in Medicine, Harvard Medical School; Senior Associate in Medicine, Peter Bent Brigham Hospital; Consultant Hematologist, Melrose Hospital, Melrose, Mass. Cloth, 344 pages, 40 figures, 5 colored plates. W. B. Saunders Co., Philadelphia, Pa.

†Experimentelle und klinische Ergebnisse mit der Friedmannschen Tuberkulosevaccine. By E. Haefliger. G. Thieme, Leipzig, 1938.

‡Vitamins and Vitamin Deficiencies. By Leslie J. Harris, Ph.D., D.Sc., F.I.C., Nutritional Laboratory, Medical Research Council and University of Cambridge. Vol. 1. Introductory and Historical. With a Foreword by Sir Frederick Gowland Hopkins, F.R.C.P., F.R.S. Washable fabric, 204 pages. 50 illustrations, $2.50. P. Blakiston's Son & Co., Philadelphia, Pa.

The chapter headings include: The Discovery of the Vitamins; The Separating and Naming of the Vitamins; Beri-Beri Vitamin B_1 Deficiency; Vitamin B_1; Nutritive Functions of Vitamin B_1.

As here told, the history of the discovery of the vitamins is of absorbing interest. After each chapter there is a brief, but meaty, summary in which the essence of the chapter is condensed, followed by a reference list applicable to the matters discussed. Complete subject and author indices are found at the end of the volume.

Those who are interested in the history of the vitamins, in the story of their development, in the methods for their detection and standardization, and in the diseases traceable to their influence—in this volume beriberi, scurvy, and rickets in particular—will find this book excellent. It is well organized, very well written, attractive in format and can be highly recommended.

Recent Advances in Medicine*

THIS book needs no introduction and in its present ninth edition presents a survey of medicine in all its varied phases. It has been extensively revised and has been largely rewritten to embody the advances of the last three years.

Not intended as a comprehensive practice, but as a reasonably comprehensive bird's-eye view of newer concepts in the field of clinical medicine, the present edition maintains the standard set by its predecessors upon which their popularity is justly based.

Symptoms and Signs in Clinical Medicine†

THIS book is complete and thorough in every respect as a text on physical diagnosis for the medical student. It is essentially clinical in character and presents the facts as simply as possible. However, at the same time the subject matter is entirely comprehensive so that it may be used also as an excellent reference by the intern and the practitioner.

In addition to descriptions for routine physical examination, there is a special section devoted to examination of sick children by Norman B. Capon, M.D., F.R.C.P. There are also chapters dealing with operations and instrumental investigations, clinical pathology, and biochemistry, as applicable to clinical medicine.

Bergey's Manual of Determinative Bacteriology‡

BERGEY'S MANUAL needs no introduction for it has been the standard reference in bacteriologic circles so long that it has become a classic.

This latest edition is greatly increased in size, showing an increase of 368 pages over the edition of 1934 and containing descriptions of 1,335 species. The book, in addition to the addition of new organisms, shows many changes and comprehensive revision.

An introductory section includes a historical survey, the rules of nomenclature, and a discussion of the identification and naming of bacteria.

As usual this is a "must" item for the bacteriologist and laboratory worker.

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*Recent Advances in Medicine. Clinical, Laboratory, Therapeutic. By G. E. Beaumont, M.D., F.R.C.P., D.P.H., Physician to the Middlesex Hospital, etc.; and E. C. Dodds, M.V.O., D.Sc., Ph.D., M.D., F.R.C.P., Coourtauld Professor of Biochemistry in the University of London, etc. Cloth, ed. 9, 431 pages, 42 illustrations, $5.00. P. Blakiston's Son & Co., Philadelphia, Pa.

†Symptoms and Signs in Clinical Medicine. An Introduction to Medical Diagnosis. By E. Noble Chamberlain, M.D., M.Sc., F.R.C.P. Ed. 2, 318 illustrations including 19 in color. William Wood & Co., and Williams & Wilkins Co., Baltimore, Md., 1938.

‡Bergey's Manual of Determinative Bacteriology. A Key for the Identification of Organisms of the Class Schizomycetes. By David H. Bergey, Formerly of the University of Pennsylvania, Philadelphia; Robert S. Breed, New York State Experiment Station (Cornell University); E. G. D. Murray, McGill University, Montreal; and A. Parker Hitchens, University of Pennsylvania. Cloth, ed. 5, 1032 pages, $10.00. Williams & Wilkins Co., Baltimore, Md.

Sex and Internal Secretions*

PERHAPS few phases of medicine have been the subject of more extensive and intensive investigation within recent years than that included in the studies of internal secretion. Ten years ago, under the guidance of the Division of Medical Sciences National Research Council and with the interest and aid of the Bureau of Social Hygiene, a study was begun on the fundamental problems of sex, the results of which are surveyed in the present volume.

Research problems and their study are always a combination of the abstract and the concrete and lead inevitably to the further problem of their ultimate correlation. To this end surveys of this character which, as it were, take stock of what has been done, are essential in evaluating results and in pointing the way to problems yet to be studied.

This volume presents a cooperative survey of recent advances in research in internal secretions and sex and as such may be taken as a comprehensive and authoritative statement of the problem as it stands today.

The book has five main sections: A. Biological Basis of Sex; B. Physiology of the Sex Glands, Germ Cells and Accessory Organs; C. Biochemistry and Assay of Gonadal Hormones; D. The Hypophysis and the Gonadotropic Hormones of Blood and Urine in Relation to the Reproductive System; and E. Additional Factors in Sex Functions and Endocrine Applications in Man.

The editors, the associate editors, and the list of contributors call the roll of the foremost investigators in this field.

This is a volume of significant importance well deserving of what has been said of its predecessors: "the most authoritative work published in any language on the endocrine aspect of sex physiology."

Clinical Bacteriology†

THIS book is essentially practical in its outlook, technical descriptions and details of cultural reactions being briefly discussed and the main emphasis placed upon the application of bacteriologic and immunologic procedures to the diagnosis, prophylaxis, and treatment of disease.

The author's style is exceedingly happy and in many ways reminiscent of Boyd in his *Pathology*. The descriptions of laboratory methods are lucid, the summaries of disease are adequate, and the discussions of treatment are such that clinical applications are at once apparent.

The organization of the book follows customary forms: classification, immunity, hypersensitivity, and general bacteriology following in that order. Theoretical discussions of immunity and hypersensitivity are brief, but their practical applications are treated at some length.

A description of each organism is followed by its cultural and biochemical activities, then by a short description of the diseases to which it has etiologic relation and then by a fairly detailed discussion of diagnosis and treatment. The human pathogens are more fully discussed than animal pathogens and nonpathogens, though all are covered.

Except for the pneumonias, the book is fully up to date. The author does not accept fully the value of serum treatment of pneumonia except in type I and II pneumococcic pneumonias. The value of sulfanilamide compounds in pneumonia is, however, discussed.

As a review of the subject for the physician in practice or in preparation for state board examinations, this book can be highly commended.

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*Sex and Internal Secretions. A Survey of Recent Research. Edited by Edgar Allen. With a Foreword by Robert M. Yerkes. Cloth, ed. 3, 1346 pages, numerous illustrations, $12.00. Williams & Wilkins Co., Baltimore, Md.

†Clinical Bacteriology. By F. A. Knott, M.D., M.R.C.P., D.P.H., Director Bacteriological Department and Lecturer in Bacteriology, Guy's Hospital. Washable fabric. 426 pages, 12 plates. P. Blakiston's Son & Co., Philadelphia, Pa.